Biochimica et Biophysica Acta 1828 (2013) 2840–2848

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Emerging role of rhomboid family proteins in mammalian biology and disease☆

Nina Bergbold, Marius K. Lemberg *

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), DKFZ-ZMBH Allianz, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 29 December 2012
Received in revised form 26 February 2013
Accepted 26 March 2013
Available online 3 April 2013

Keywords:
Regulated intramembrane proteolysis
Pseudoenzyme
Protein trafficking
Epidermal growth factor receptor signaling
ADAM17
Endoplasmic reticulum-associated protein degradation

ABSTRACT

From proteases that cleave peptide bonds in the plane of the membrane, rhomboids have evolved into a heterogeneous superfamily with a wide range of different mechanistic properties. In mammals 14 family members have been annotated based on a shared conserved membrane-integral rhomboid core domain, including intramembrane serine proteases and diverse proteolytically inactive homologues. While the function of rhomboid proteases is the proteolytic release of membrane-tethered factors, rhomboid pseudoproteases including iRhoms and derlins interact with their clients without cleaving them. It has become evident that specific recognition of membrane protein substrates and clients by the rhomboid fold reflects a spectrum of cellular functions ranging from growth factor activation, trafficking control to membrane protein degradation. This review summarizes recent progress on rhomboid family proteins in the mammalian secretory pathway and raises the question whether they can be seen as new drug targets for inflammatory diseases and cancer. This article is part of a special issue entitled: Intramembrane Proteases.

Contents

Abbreviations: EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERAD, ER-associated degradation; IRHD, iRhom homology domain; iRhom, inactive rhomboid; L1, loop 1; LPS, lipopolysaccharide; RIP, regulated intramembrane proteolysis; RHBDL, rhomboid-like protein; TNFα, tumor necrosis factor α; TM, transmembrane

☆ This article is part of a special issue entitled: Intramembrane Proteases.

* Corresponding author. Tel.: +49 6221 545889.
E-mail address: m.lemberg@zmbh.uni-heidelberg.de (M.K. Lemberg).

0005-2736/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2013.03.025

1. Introduction

Rhomboids were first discovered in *Drosophila melanogaster*, where they act as key regulators of epidermal growth factor receptor (EGFR) signaling [1,2]. By a specific cut in the transmembrane (TM) anchor, *Drosophila* rhomboid proteases trigger the activation and secretion of three growth factors called Spitz, Gurken and Keren [3–5]. Moreover, *Drosophila* Rhomboid-1 became the founding member of a universally conserved class of intramembrane serine proteases that fulfill a wide range of functions ranging from regulated intramembrane proteolysis (RIP) to degradation (see related reviews in this issue [6–8]). Extensive mutagenesis studies and biochemical assays revealed that rhomboid proteases use a catalytic histidine–serine dyad between conserved TM segments of their polytopic rhomboid core domain to cleave their substrates (Fig. 1) [4,9–12]. Invariant additional features include a tryptophan–arginine (WR) motif found in loop 1 (L1) and an Engelman helix dimerization motif (GxxxG) in TM segment 6. Several crystal structures of the bacterial rhomboid protease GlpG solved to atomic resolution revealed that the membrane-integral active site is formed by a six-TM helix bundle, which is clamped sidewise by an unusual L1 structure that resides in the upper leaflet of the lipid bilayer (for recent review see [13]). Although still no physiological substrate is known, mechanistically the *Escherichia coli* rhomboid GlpG is the best-studied rhomboid protease. In addition, the GlpG fold became the structural reference for an emerging class of catalytically inactive homologues (Fig. 1) [14–16]. These so-called rhomboid pseudoproteases, which include derlins and iRhoms (for 'inactive rhomboid'), bind proteins within the eukaryotic secretory pathway and control their fate by either regulating their trafficking or degradation. Emerging functional parallels between the rhomboid proteases and their catalytically inactive cousins are that they share the way how they interact with proteins in the plane of the membrane—either to trigger their release by a proteolytic cut or to hand them over to different cellular factors.

While by the help of powerful genetics the function of *Drosophila* rhomboids has been resolved, still much less is clear about the function of the mammalian homologues. Even more challenging is the analysis of proteolytically inactive rhomboid homologues. However, recent work, including the first reports on knockout mice, shows that mammalian rhomboid family proteins affect a number of different processes such as intercellular signaling, wound healing and protein degradation. In this review we discuss the current knowledge about the function of rhomboid family proteins in the mammalian secretory pathway and ask whether they can be seen as new targets for therapeutic intervention in human diseases. The emerging function of the mitochondrial rhomboid protease PARL in organelle homeostasis and Parkinson's disease is discussed in an accompanying review in this issue [6].

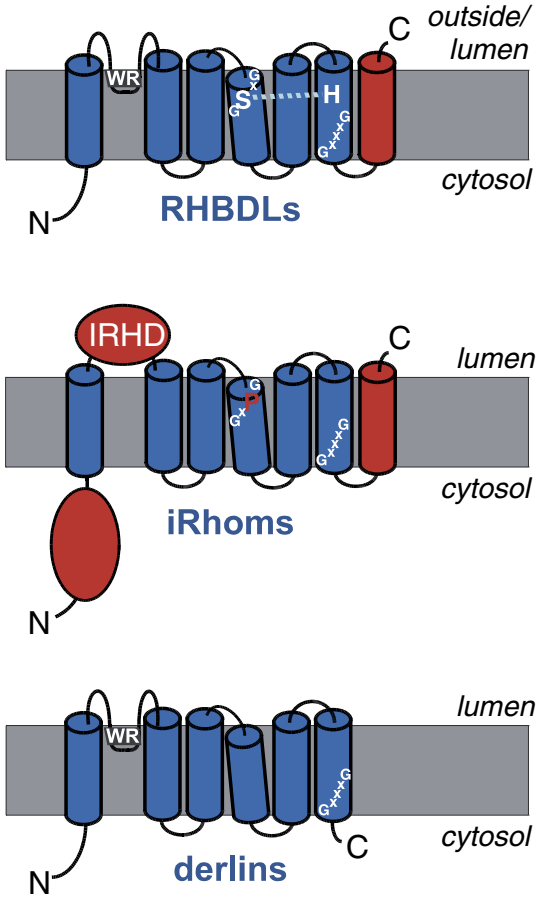

Fig. 1. Mammalian rhomboid family proteins share a membrane-integral core domain. Topology models for human rhomboid proteases (RHBDLs), iRhoms and derlins with the conserved six-pass TM domain core in blue. Structurally important features such as the Engelman TM helix–helix dimerization motif (GxxxG) and the L1 structure extending sidewise into the membrane (WR) are shown. Extra domains including the IRHD are highlighted in red. Of note, RHBDL4 and PARL lack the seventh C-terminal TM helix (highlighted in red) and have instead additional domain fusions (not shown). For RHBDLs the protease active site motifs 'GxSG' and 'H' form a catalytic dyad between TM helices 4 and 6, whereas iRhoms have a 'GPxG' sequence.

2. Conserved membrane-integral core domain defines the rhomboid superfamily

Many genes have been annotated as rhomboid proteases based on sequence similarity. This bioinformatic search also revealed several close homologues lacking key catalytic residues, which are predicted to be proteolytically inactive [15,17]. The Pfam protein family database further includes the more distant homologues such as derlins and the so far uncharacterized protein TMEM115, forming an extended superfamily of rhomboid-like proteins [18]. Currently 14 rhomboid family proteins are known, five rhomboid proteases and nine catalytically inactive homologues (Table 1). Phylogeny suggests that the so-called pseudoproteases have evolved by several independent gene duplication events of proteolytic rhomboids, followed by diversification and loss of activity (Fig. 2) (see recent review [19]). All members of the rhomboid superfamily share significant sequence conservation within a core of six TM domains, suggesting that their membrane-integral part shows structural similarities (Fig. 1). Consistent with this, human Derlin-1 has six TM segments, and not four as initially suggested, and structure homology modeling predicts that it shares with *E. coli* GlpG the rhomboid fold [16]. Relationship between distant family members and the bona fide rhomboid proteases best can be seen by the conservation of characteristic signatures such as the L1 'WR' motif or the 'GxxxG' helix dimerization motif in TM segment 6 (Table 1). A recent thermodynamic analysis of GlpG showed that the rhomboid-fold is primarily formed by four discrete regions including the 'WR' and 'GxxxG' motifs, which by both packaging and hydrogen bonding stabilize the structure of the protease [20]. Although not all these 'keystones' are strictly conserved for all rhomboid pseudoproteases, the high sequence similarity suggests that the entire rhomboid superfamily shares the same protein fold and structural flexibility. In a striking variability, most rhomboid proteases have evolved additional TM helices and different globular domains that are fused to the N- and C-terminus or extended loop insertions (Fig. 1) [15,17]. Assuming that rhomboids have evolved from a common ancestor with the basic six-TM domain structure, this makes the rhomboid superfamily to a rare case where topology has evolved by attachment of a single nonhomologous TM segment [15], instead of a more typical internal gene duplication event [21].

Table 1
Characteristics of mammalian rhomboid family proteins.

Family member	Localization	Structural signatures	Activity	Function
RHBDL1/*veinlet-like 1*	Golgi	WR (L1) GxSG (TMD4)GxxxG (TMD6)	*Protease* (*predicted*)	?
RHBDL2	Plasma membrane	WR (L1)GxSG (TMD4)GxxxG (TMD6)	Protease	RIP, abundance control
RHBDL3/*veinlet-like 4*	Endosomes	WR (L1)GxSG (TMD4)GxxxG (TMD6)	*Protease* (*predicted*)	?
RHBDL4/RHBDD1	ER	WQR (L1)GxSG (TMD4)GxxxG (TMD6)	Protease, ubiquitin-binding, p97-recruitment	ERAD
PARL	Mitochondrial inner membrane	GxSG (TMD5)GxxxG (TMD7)	Protease	RIP, abundance control
iRhoms (1, 2)	ER—Golgi	GPAG (TMD4)GxxxG (TMD6)	Pseudoprotease	Trafficking control, ERAD?
Derlins (1, 2, 3)	ER	WR (L1)GxxxG (TMD6)	Pseudoprotease	ERAD
UBAC2	ER	WR (L1)GxxxG (TMD6)	*Pseudoprotease*, *ubiquitin-binding* (*predicted*)	ERAD
RHBDD2	Golgi	YR (L1)GxxxG (TMD6)	*Pseudoprotease*, (*predicted*)	?
RHBDD3	?	HR (L1)GxxxG (TMD6)	*Pseudoprotease*, *ubiquitin-binding* (*predicted*)	?
TMEM115	?	WT (L1)GxxxG (TMD6)	*Pseudoprotease*, (*predicted*)	?

3. Mammalian rhomboid proteases

The first gene of a mammalian rhomboid protease was cloned in 1998 when *Drosophila* Rhomboid-1 still was seen as a mysterious activator of growth factor secretion and awaited its discovery as intramembrane protease [22]. In analogy to the fly gene, this protein of unknown function was named *RHBDL1* (for 'rhomboid-like protein 1'). RHBDL2, the second mammalian rhomboid, later was shown to share the activity of *Drosophila* Rhomboid-1 to cleave Spitz, whereas RHBDL1 did not show any activity [4]. Currently, five rhomboid proteases are known in mammals that scatter across all major cellular organelles and are predicted to have various different functions (Table 1) [15]. Fluorescence microscopy suggests that ectopically expressed RHBDL1, like *Drosophila* Rhomboid-1, is a Golgi enzyme, whereas RHBDL2 is found at the plasma membrane and RHBDL3 show a punctuated pattern characteristic for endosomal structures [23]. RHBDL4 initially has been suggested to be a mitochondrial protein (also referred to as RHBDD1) [24], whereas our more recent subcellular fractionation and fluorescence microscopy analysis overcame this view revealing that RHBDL4 localizes to the endoplasmic reticulum (ER) [25]. In contrast, PARL is a true mitochondrial rhomboid protease that localizes to the inner mitochondrial membrane [26,27].

3.1. Substrate identification: key for functional characterization of rhomboid proteases in the late secretory pathway

Rhomboid proteases initially have been thought to cleave only type I membrane proteins. Recently several exceptions from this rule have been described, suggesting that the substrate spectrum of rhomboid proteases is broader than initially anticipated [25,28–30]. For the plasma membrane-localized RHBDL2 several substrates have been identified by candidate testing, whereas for the other rhomboid proteases in the late secretory pathway no substrates are known yet (see below and Table 2). Since it has been suggested that RHBDL2 requires proteolytic cleavage within L1 to become activated [31], the question arises whether rhomboids are synthesized as zymogens and if therefore upon ectopic expression of RHBDL1 and -3 no activity was recorded. A number of functional assays, however, suggest that rhomboids are constitutively active enzymes and differences in the substrate specificity may explain the apparent lack of activity of certain RHBDLs. As for classical soluble proteases, rhomboids need to be tightly controlled in order to prevent damage to the membrane [32]. Because of the biophysical properties of the lipid bilayer, substrate recognition of rhomboid proteases follows different principles than for soluble enzymes [33]. For rhomboid proteases, two different models for substrate recognition have been suggested. In the first model, the conformational flexibility of the substrate peptide backbone combined with immersion of the membrane in the vicinity of the rhomboid active site is sufficient to provide specificity [34,35]. For the well-characterized *Drosophila* substrate Spitz, a glycine–alanine motif has been shown to serve as a helix break that allows unfolding of the TM domain into the rhomboid active site [34]. The second model suggests that rhomboid proteases primarily recognize a specific sequence surrounding the cleavage site, and that TM helix-destabilizing residues are a secondary feature required for some substrates only [36,37]. Although a recent paper challenges

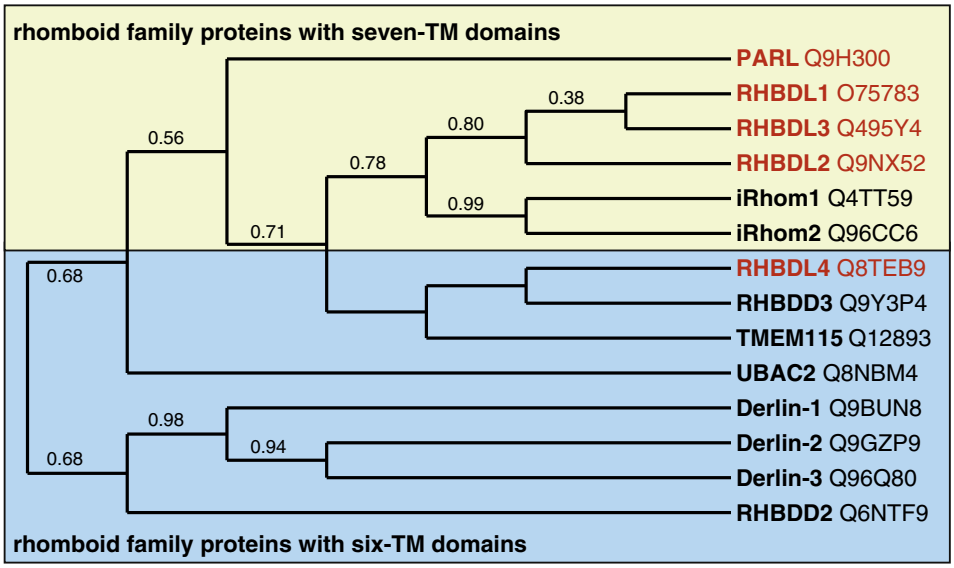

Fig. 2. Phylogeny of human rhomboid family proteins in the secretory pathway. Phylogenetic relationship of rhomboid proteases (highlighted in red) and catalytically inactive pseudoproteases (black) based on the conserved regions (L1, TM domains 2, 4, and 6) as determined previously [15]. Swissprot accession numbers are indicated.

Table 2
Substrates of mammalian rhomboid proteases.

Family member	Substrates	References
RHBDL1/*veinlet-like 1*	–	
RHBDL2	*Drosophila* Spitz	[3]
	Drosophila Gurken	[5]
	Thrombomodulin	[23,41]
	Ephrin-B2 & -B3	[43]
	ProEGF	[52]
	ErbB-1	[53]
RHBDL3/*veinlet-like 4*	–	
RHBDL4/RHBDD1	BIK1	[24]
	TSAP6	[30]
	PreTCRα, TCRα, MPZ-L170R, opsin—degron, polycystin-1 truncations	[25]
	Drosophila Spitz	[35]
PARL	OPA1	[91]
	Omi/HtrA2	[92]
	PINK1	[93–96]
	PGAM5	[97]

this view [35], we may speculate that RHBDLs are controlled by sequence specificity. Substrate recognition probably is modulated by additional features ranging from trafficking signals to helical secondary structure elements, which prevents access to potential rhomboid protease consensus sites.

3.1.1. RHBDL2 is important for wound healing

The first known physiological substrate of a mammalian rhomboid protease was the cell-surface protein thrombomodulin [23], which serves as high-affinity thrombin receptor to modulate blood coagulation [38]. In addition thrombomodulin has various other functions including the control of cell adhesion and migration in processes such as wound healing [39]. Several proteases have been implicated in thrombomodulin shedding and soluble forms were detected that served as indicator for endothelial damage [40]. Database mining for Spitz-type TM substrate features combined with candidate testing showed that ectopically expressed RHBDL2 cleaves thrombomodulin [23]. Mutagenesis and domain swap experiments, however, revealed that the Spitz-type substrate motif was an ancillary feature and instead the cytosolic thrombomodulin tail was essential and sufficient to drive RHBDL2-catalyzed cleavage [23]. Later work on the substrate specificity showed that thrombomodulin fit the cleavage site specificity observed for bacterial rhomboids, supporting the hypothesis that certain rhomboid proteases are controlled primarily by sequence specific substrate recognition [37]. Physiological relevance of RHBDL2-catalyzed thrombomodulin shedding was recently provided by an in vitro wound-healing assay [41]. This report of endogenous RHBDL2 activity in thrombomodulin processing demonstrated its crucial role in remodeling of cell–cell contact points. Although the soluble thrombomodulin ectodomain acts as a mitogen [42], and it efficiently promotes wound healing, it is not clear whether RHBDL2 activity also plays a direct role in remodeling of cell–cell contact points (Fig. 3) [41]. Further work including studies in RHBDL2 knockout mice will be required to resolve the underlying molecular events and the physiological relevance of RHBDL2 activity in wound healing.

3.1.2. RHBDL2 cleaves the cell-adhesion and signaling molecule ephrin

Further work showed that ectopically expressed RHBDL2 cleaves ephrin-B3 and to a lower extend ephrin-B2 [43]. This cleavage was dependent on a Spitz-type TM helix break and is not affected by the cytosolic substrate tail. Ephrins are a heterogeneous protein family that by binding to cognate ephrin receptors modulate cell motility and adhesion [44]. In addition to driving bidirectional signaling via tyrosine phosphorylation, receptor binding has been observed to trigger proteolytic processing of various different ephrins [45–47]. Although the molecular details of these shedding events in most circumstances are not completely clear yet, ADAM-catalyzed cleavage of ephrin-A2 has been shown to control axonal repulsion by terminating high-affinity cell–cell contact points [45]. Whether RHBDL2-catalyzed cleavage of ephrins-B2 and -3 similarly is involved in remodeling of cell–cell contacts remains to be addressed.

3.1.3. Noncanonical activation of EGF by RHBDL2

Like the *Drosophila* growth factors, also mammalian EGFR ligands are synthesized as membrane-tethered precursors and are only active upon proteolytic release [48]. Despite being the trigger of EGFR signaling in *Drosophila*, until recently, it was a commonly accepted view that mammalian rhomboid proteases are not involved. Instead, there is compelling evidence that ADAM proteases are key for the activation of mammalian EGFR ligands [48–50]. For some EGFR ligands such as proEGF, however, also other proteases such as kallikreins contribute to proteolytic processing [51]. Recent work showed that in several tumor cell lines endogenous RHBDL2 is able to cleave and activate proEGF, whereas no other RHBDL showed activity on this substrate [52]. The TM domain of proEGF has no Spitz-type helix-break, corroborating that RHBDL2 can recruit its substrate by alternative mechanisms. As had been observed for other rhomboid proteases, proEGF is cut by RHBDL2 just outside the TM domain. Interestingly, like for thrombomodulin, also the cleavage site of proEGF fits the consensus of bacterial rhomboid proteases [37,52]. Taken together with the lack of any helix-breaking residue in the TM domain, this suggests that for proEGF sequence specificity provides the main layer of control. Mutation of the rhomboid cleavage site or RHBDL2 knockdown both had no effect on ADAM protease dependent EGF shedding, indicating that the rhomboid- and metalloprotease-dependent pathways act in parallel rather than serially [52]. Whether RHBDL2 plays a crucial role in EGFR signaling in vivo and whether its inhibition offers a new therapeutic intervention in cancer will be interesting questions to be addressed in the future.

3.1.4. Does RHBDL2 affect receptor down-regulation?

Another link of RHBDL2 to modulation of EGFR signaling is provided by the identification of EGFR, also known as ErbB-1, as its fourth substrate [53]. EGFR/ErbB-1 activity is modulated by several mechanisms including its ubiquitin-mediated endocytosis, which ensures termination

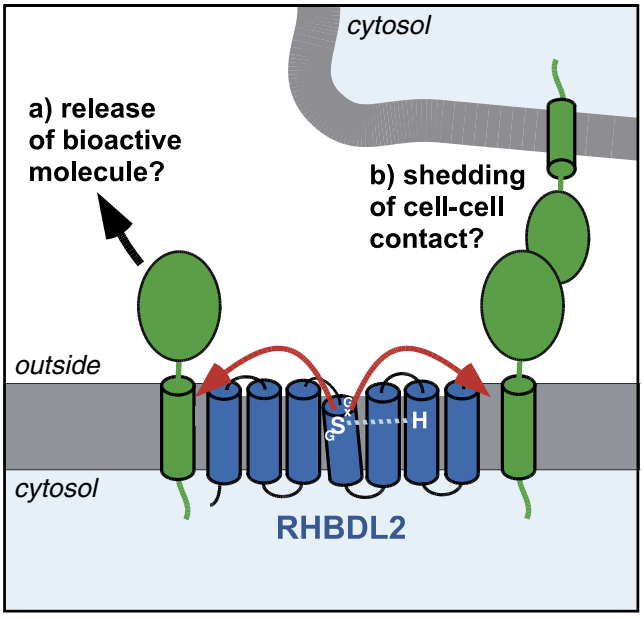

Fig. 3. Putative role of RHBDL2 in the release of bioactive molecules and remodeling of cell–cell contact points. The plasma membrane-localized RHBDL2 cleaves the type I membrane protein thrombomodulin. The function of RHBDL2 in wound healing may be the release of soluble bioactive ectodomain (a), or the regulation of cell adhesion by shedding of anchoring points (b).

of receptor signaling by lysosomal degradation [54]. An alternative inactivation mechanism may be provided by ligand-induced shedding of ErbB-1. Although for the related receptor tyrosin kinase ErbB-4, ADAM protease and γ-secretase catalyzed RIP have been reported by several laboratories [55–57], just recently ectopically expressed RHBDL2 has been shown to cleave ErbB-1 [53]. Rhomboid-catalyzed ErbB-1 processing is stimulated by ligand-binding and ionomycin-induced calcium signaling, suggesting that shedding is part of the physiological receptor downregulation. Like for all other RHBDL2 substrates, upon cleavage the ectodomain of ErbB-1 is released into the extracellular space. Interestingly, following RHBDL2-catalyzed cleavage, the C-terminal fragment remains predominantly in the membrane fraction and only a minor portion was observed in the nucleus [53]. This is strikingly different to γ-secretase catalyzed intramembrane cleavage of ErbB-4, which leads to an efficient release and nuclear accumulation of a transcriptionally active intracellular domain [57]. Although the RHBDL2-cleavage site in ErbB-1 has not been determined yet, this difference suggests that shedding by RHBDL2 occurs in the juxtamembrane region and leaves the TM anchor intact. Whether RHBDL2 acts similarly to ADAM sheddase and γ-secretase to trigger signaling to the nucleus or whether RHBDL2 only inactivates cell surface receptors remains to be studied. From a mechanistic point of view, RHBDL2-catalyzed cleavage of ErbB-1 is the intriguing first report of a ligand-dependent rhomboid substrate cleavage [53].

3.1.5. In search of RHBDL1 and RHBDL3 substrates

Although ectopic expression of RHBDL1 and RHBDL3 leads to partial colocalization with RHBDL2 substrates [23], so far no significant proteolytic activity has been reported. Since both proteases show all essential active site residues (see Table 1), we may envision that they are active rhomboid proteases but show a different substrate spectrum than RHBDL2 and *Drosophila* Rhomboid-1. Therefore, more potent methods for substrate identification are urgently needed to decipher the function of RHBDL1 and RHBDL3. Expression profiling suggests that RHBDL3 plays a role in pancreas development [58]. More recently, a microarray study in post mortem human brains revealed a correlation between *RHBDL3* gene expression and chronological aging, suggesting that this rhomboid activity is causally related to the aging phenotype [59]. However, this remains speculative until substrates of RHBDL3 are identified and its activity in aging cells has been measured.

3.2. RHBDL4 promotes ER-associated degradation

Consistent with its distinct phylogenetic origin, RHBDL4 is substantially different from the three other rhomboid proteases in the secretory pathway. By lacking the seventh TM domain, which is a characteristic feature of eukaryotic rhomboid proteases, it possibly resembles a close relative of an ancestral rhomboid common to all kingdoms of life [15]. Another surprising finding was that RHBDL4 localizes to the ER [25], which is commonly assumed to be a protease free zone. This makes sense, since the major function of the ER is protein folding [60], and not fully matured proteins have to be protected from inappropriate proteolysis. Despite that, misfolded proteins have to be efficiently removed from the ER, in order to avoid accumulation of toxic aggregates. Consistent with its localization, RHBDL4 is part of the ER-associated protein degradation (ERAD) machinery [25]. Although the molecular details of ERAD have not been fully resolved yet, work by a number of labs showed that aberrant proteins are usually dislocated from the ER into the cytosol as full-length proteins where they become degraded by the ubiquitin proteasome system [61]. However, RHBDL4-triggered degradation is different, leading to clipping of ERAD substrates already in the ER membrane. Interestingly, RHBDL4 can also cleave within luminal loops and TM regions of polytopic membrane proteins (Table 2) [25,30]. ER localization posed the question how RHBDL4 is regulated in the absence of controlled substrate trafficking. An unexpected twist to rhomboid biology is that RHBDL4 is the first intramembrane protease that specifically recognizes substrate ubiquitination by a cytosolic ubiquitin-interacting motif (Fig. 4) [25]. This is functionally linked to the canonical ERAD pathways since ubiquitination of RHBDL4 substrates is dependent on E3 protein ligases such as gp78. Different to all previously characterized rhomboid proteases, RHBDL4-generated cleavage products are not secreted [25]. Instead, luminal fragments are retained in the ER, dislocated into the cytosol and finally degraded by the proteasome. The nature of the protein-conducting channel that mediates passage of ERAD substrates and their cleavage fragments through the membrane is not known. However, we observed that RHBDL4 recruits the AAA+-type ATPase p97 in order to use the chemical energy of ATP to drive this reaction (Fig. 4). Ablation of RHBDL4 activity leads to proteotoxic stress, indicating that clipping of ERAD substrates plays a vital role in the control of ER protein homeostasis [25]. The questions why RHBDL4 acts parallel to classical ERAD dislocation pathways, and what the functional benefit of substrate clipping in the plane of the ER membrane is, remain to be addressed. Of note, alternative RHBDL4 functions such as roles in apoptosis regulation [24] and exosome biosynthesis [30] have been suggested (see Table 2 for putative substrates).

4. Rhomboid pseudoproteases

A growing number of proteins are recognized as rhomboid family members despite being predicted to be proteolytically inactive [15–17]. Although the molecular understanding of these pseudoproteases is still at the beginning, the evolutionary relationship and predicted structural parallels to the rhomboid fold indicate that they share mechanistic features of how they interact with their protein clients. The functional outcome and the fate of membrane-bound proteins, however, are unrelated and catalytically inactive rhomboid family members affect a wide range of different cellular pathways.

4.1. iRhoms

A striking sequence relationship exists between rhomboid proteases and a tightly clustered group of homologues called iRhoms

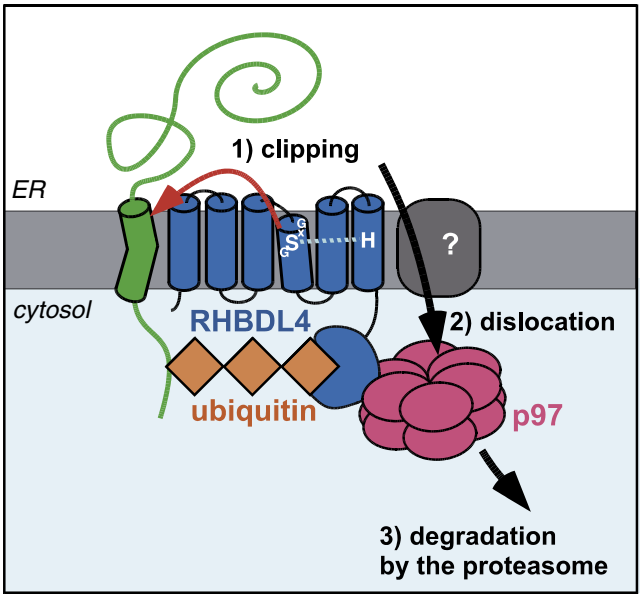

Fig. 4. RHBDL4 promotes degradation of membrane proteins. ER-resident RHBDL4 cleaves unstable membrane proteins in an ubiquitin dependent manner (1). Cleavage fragments are dislocated into the cytosol (2) dependent on the AAA+-type ATPase p97 and are finally degraded by the cytosolic proteasome (3).

(Fig. 2) [15,17]. These pseudoproteases are found throughout the metazoan kingdom. However, since in the nematode *Caenorhabditis elegans* both iRhom paralogues show an apparent intact catalytic dyad [15], initially it was unclear whether they are proteases or not. Strikingly, all iRhoms have a proline in the x-position of the apparent 'GxSG' catalytic motif, suggesting that the rhomboid fold is significantly altered and they have evolved a nonproteolytic function (Fig. 1) [15]. Consistent with this hypothesis, the related proline mutation kills the proteolytic activity of rhomboid proteases and all iRhoms tested did not cleave any known rhomboid substrates [62]. In addition, iRhoms show two specific features that are not found in any active rhomboid protease (Fig. 1). First, iRhoms show an extended cysteine-rich loop between TM domains 1 and 2, which because of its high sequence conservation is called iRhom homology domain (IRHD) [15]. Second, the conserved cytosolic N-terminal domain harbors several potential phosphorylation sites suggesting a regulatory role [63]. The function of these iRhom-specific features is not known, however, the high degree of sequence conservation taken together with putative gain-of-function mutations within the IRHD and the N-terminus underscores their importance [64–66].

4.1.1. iRhom regulates activation of the sheddase ADAM17

Although iRhoms are not active proteases, their physiological function is linked to proteolytic ligand release. Two recent studies observed that iRhom2 knockout mice injected with lipopolysaccharide (LPS), to mimic a bacterial infection, showed reduced levels of secreted tumor necrosis factor α (TNFα) [64,67]. As a consequence of the resulting immune suppression, iRhom2-deficient mice failed to control a bacterial infection with *Listeria monocytogenes* [64]. This is because in macrophages, which are cells that secrete TNFα, iRhom2 acts as an essential ER to Golgi trafficking factor for the metalloprotease ADAM17 (also known as TACE) [64,67,68]. In the Golgi, ADAM17 is activated like many other proteases in the secretory pathway [69], which in turn is an essential step for ADAM17-catalyzed release of TNFα [64,67,68]. Thereby iRhom2-controlled maturation defines a new layer of ADAM17 regulation in macrophages. Interestingly, in a recent publication it has been shown that iRhom1, which in contrast to iRhom2 is ubiquitously expressed, is responsible for ADAM17 maturation in other cells than macrophages [70]. Consistent with a role in the immune response, two main activators of inflammatory arthritis were shown to stimulate iRhom2/ADAM17-dependent cleavage of TNFα. Since iRhom2 expression is restricted to immune cells, its inactivation will not influence ADAM17 functions in other cell-types, which makes iRhom2 a potential drug target for inflammatory diseases [70]. Related to these findings, a recent report showed that iRhom2 is expressed and transcriptionally upregulated in hepatocytes exposed to LPS [63]. Here iRhom2 participates in ADAM17-catalyzed down-regulation of the TNF receptor, which protects hepatocytes from TNFα-induced apoptosis. Taken together with the flurry of reports that iRhom2 activates TNFα activity, this revealed ADAM17 trafficking as an important regulator, and if drugable, iRhom2 as a selective and potential target for the treatment of inflammatory diseases.

4.1.2. iRhom in the abundance control of EGFR ligands

Another function of iRhoms has been observed in *Drosophila*, where it inhibits EGFR signaling in the nervous system [62]. Different to an initial hypothesis that the iRhom pseudoprotease directly blocks active rhomboid proteases [17], it reduces the level of growth factor substrates by triggering their degradation (Fig. 5) [62]. Consistent with a link of iRhom to the ERAD pathway, *Drosophila* iRhom genetically interacts with the E3 ubiquitin ligase Hrd1 and the ERAD substrate receptor EDEM [62]. In the same study, also mammalian iRhoms have been shown to trigger ERAD of EGFR ligands [62]. The emerging picture is that secretion of EGFR ligands is a highly dynamic and regulated process and that iRhoms act as negative regulators of EGFR ligand trafficking. This suggests that iRhoms have bivalent

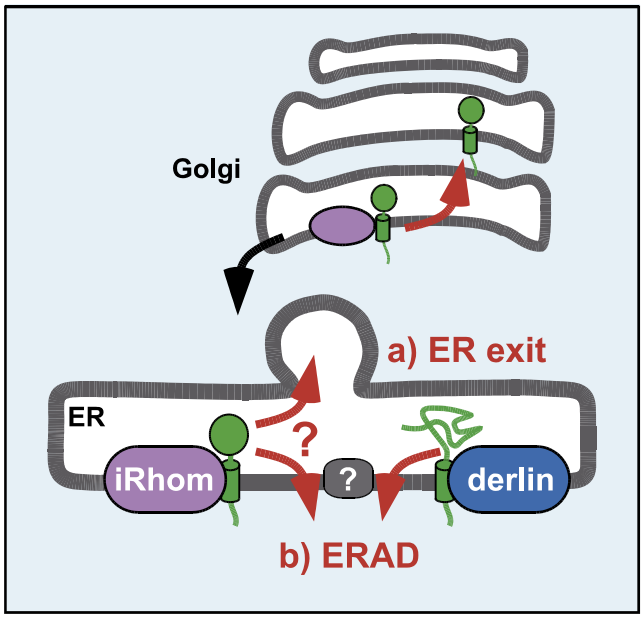

Fig. 5. Role of Rhomboid pseudoproteases in the control of ER protein homeostasis. Whereas iRhom binds its clients in the ER and guides them along the secretory pathway to the Golgi (a), derlins are bona fide ER proteins that in concert with other ERAD factors determine their substrates for dislocation into the cytosol (b). By a mechanism that has not been determined yet, certain iRhom clients are retained in the ER and funneled into the ERAD pathway (b).

functions; either promoting trafficking to the Golgi, or blocking ER export of the ligand, which leads to its turnover by the ERAD pathway (Fig. 5) [62,64,67]. Currently it is not clear how iRhom makes the decision whether its clients are exported or degraded. We may speculate that iRhom regulates the ER export rate, the canonical ER quality control and client properties such as N-linked glycans determine the extent to which the client is degraded [71].

4.1.3. iRhoms implicated in cancer

With a potential to affect the cellular activity of two main players of the EGFR pathway, the ADAM17 sheddase and EGFR ligands, iRhom emerges as a master regulator, suggesting that its expression level has to be tightly controlled [62,64,67]. Consistent with such an important role in EGFR signaling, it has been shown that misregulated iRhom levels affect cancer development and tumor growth [66,72,73]. Although the underlying mechanisms have not been resolved yet, high throughput sequencing revealed that missense mutations in the *iRhom2* gene cause tylosis with esophageal cancer and other *iRhom2* mutations may play a role in ovarian cancer [65,66,74]. The hyperproliferative phenotype observed in the esophageal cancer cells implies that altered iRhom2 levels lead to sustained EGFR signaling [66]. A link of iRhoms to EGFR signaling in humans is further supported by the fact that iRhom1 physically interacts with transforming growth factor α [75]. Moreover, siRNA-mediated iRhom1 knockdown in tissue culture cells has been described to diminish EGFR transactivation, which is the crosstalk between G-protein coupled receptors and the EGFR [73]. The physiological relevance of this link to EGFR transactivation, however, remains to be investigated. In conclusion, both mammalian iRhom paralogues have been linked by several lines of evidence to key signaling pathways, suggesting that tuning their activity may be a promising new therapeutic strategy for disease intervention.

4.2. Derlins

More distant rhomboid family proteins are derlins (Fig. 2) [12,16]. The first example Der1 (for 'degradation in the ER') was identified by

a genetic screen in *Saccharomyces cerevisiae* for ERAD factors [76]. Later, it was shown to interact with the E3 ubiquitin ligase Hrd1 and the AAA+-type ATPase Cdc48 (known as p97 in mammals) in order to extract aberrant luminal and membrane-anchored proteins [77,78]. Likewise, human derlins were shown to be central ERAD factors. Derlin-1 was identified by two independent mass spectrometry-based approaches probing the environment of factors involved in ERAD [79,80]. Subsequently, Derlin-1 was shown to act in a multiprotein network that mediates ERAD of a heterogeneous set of proteins including disease-associated mutants of the polytopic membrane protein CFTR (for 'Cystic fibrosis transmembrane conductance regulator') [78,81–83]. Likewise, the two other human paralogues, namely Derlin-2 and -3, are involved in ERAD [82,84]. The molecular function of derlins is still unclear and it is not known what determines the specificity between derlin paralogues. Although they have been suggested to form a protein-conduction pore for 'retrotranslocation' of ERAD substrates, a recent revision of their TM topology and a structure homology model indicate that they have a rhomboid fold instead [16]. Intriguingly, the characteristic 'WR' and 'GxxxG' motifs (see Fig. 1), which are both crucial for rhomboid protease activity and stability, are also key for Derlin-1-mediated protein dislocation [16]. Assuming that the rhomboid superfamily share the way they interact with substrates and clients, derlins may serve as receptors for ERAD substrates that use the membrane-perturbing action of the rhomboid domain to facilitate dislocation into the cytosol (Fig. 5) [61,71].

4.3. New emerging catalytically inactive rhomboid family proteins

Based on sequence comparison, several so far functionally uncharacterized proteins are predicted to belong to the rhomboid superfamily (Fig. 2). Since these more distant rhomboid family proteins show clear differences to rhomboid protease signature (Table 1), it remains to be investigated whether they are all structurally related. The limited overall sequence conservation suggests that these putative rhomboid pseudoproteases fulfill different functions. UBAC2 (for 'ubiquitin-associated domain-containing protein 2') has been shown by a proteomics and functional genomics study to serve as an ERAD factor [82]. This is supported by the fact that UBAC2 is upregulated upon ER stress, suggesting a vital role in the control of ER protein homeostasis. More recently, UBAC2 has been shown to restrict trafficking of the adipose triglyceride lipase regulator UBXD8 from the ER to lipid droplets, indicating a link to the control of cellular lipid storage [85]. How UBAC2 exactly interplays with the ERAD pathway and regulates lipid droplet turnover, however, remains to be investigated. The function of three other highly conserved rhomboid pseudoproteases, namely RHBDD2, RHBDD3 and TMEM115 (Table 1), is not known. For Golgi-resident RHBDD2, a missense mutation in the *RHBDD2* gene hints to a role in the onset of retinitis pigmentosa [86]. Furthermore, elevated expression levels in breast and colorectal cancer indicate a role in tumorigenesis [87–89]. Not only because of their clinical relevance, it will be interesting to reveal the function and the molecular mechanism of these just emerging classes of rhomboid family proteins.

5. Concluding remarks

After the discovery that rhomboids are intramembrane proteases, progress on the mammalian homologues was initially slow. Although they can be inhibited with a broad-spectrum serine protease inhibitor dichloroisocoumarin [4,11,12], the short half live and toxicity limit the applicability of this compound, still making the analysis of endogenous rhomboid activity challenging. A recent in vitro drug screen with bacterial rhomboid proteases identified β-lactams as potential lead compound that also inhibit RHBDL2 [90], raising hopes that in the future more potent and selective rhomboid protease inhibitors may find application as therapeutic drugs [33]. Moreover, several new substrates for mammalian rhomboid proteases have been identified and first reports have started to illuminate the function of catalytically inactive homologues. Although still several putative rhomboid pseudoproteases have not been functionally characterized, it already becomes clear that the rhomboid superfamily affects the fate of membrane proteins in many biological and medical important contexts. Inevitably, the recent work has also posed new questions. Among them, a fundamental unsolved problem is how rhomboid proteases and their catalytically inactive cousins specifically interact with substrates and clients. Moreover, we still struggle in finding endogenous substrates and clients. In order to allow physiological conclusions, more efficient and unbiased methods for substrate identification are urgently needed. Together with a detailed functional characterization of the rhomboid superfamily, this will likely help to reveal new mechanisms important for human biology and disease. Since rhomboid family proteins are key players of various important biological pathways, which when disturbed can lead to inflammatory diseases and cancer, likely it will be just a matter of time until the first drugs are targeting this class of proteins.

Acknowledgements

We thank Dönem Avci and Cathrin Meissner for critical reading of the manuscript. This work is supported by the Deutsche Forschungsgemeinschaft (SFB 1036, TP 12).

References

[1] M.A. Sturtevant, M. Roark, E. Bier, The Drosophila rhomboid gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway, Genes Dev. 7 (1993) 961–973.
[2] M. Freeman, The spitz gene is required for photoreceptor determination in the Drosophila eye where it interacts with the EGF receptor, Mech. Dev. 48 (1994) 25–33.
[3] J.R. Lee, S. Urban, C.F. Garvey, M. Freeman, Regulated intracellular ligand transport and proteolysis control EGF signal activation in Drosophila, Cell 107 (2001) 161–171.
[4] S. Urban, J.R. Lee, M. Freeman, Drosophila rhomboid-1 defines a family of putative intramembrane serine proteases, Cell 107 (2001) 173–182.
[5] S. Urban, J.R. Lee, M. Freeman, A family of rhomboid intramembrane proteases activates all Drosophila membrane-tethered EGF ligands, EMBO J. 21 (2002) 4277–4286.
[6] Y.L. Chan, G.A. McQuibban, The mitochondrial rhomboid: its rise from obscurity to the pinnacle of disease-relevant genes, Biochim. Biophys. Acta 1828 (2013) 2916–2925.
[7] P. Rather, Role of rhomboid proteases in bacteria, Biochim. Biophys. Acta 1828 (2013) 2849–2854.
[8] D.L. Sibley, The roles of intramembrane proteases in protozan parasites, Biochim. Biophys. Acta 1828 (2013) 2908–2915.
[9] K. Esser, B. Tursun, M. Ingenhoven, G. Michaelis, E. Pratje, A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1, J. Mol. Biol. 323 (2002) 835–843.
[10] S. Urban, D. Schlieper, M. Freeman, Conservation of intramembrane proteolytic activity and substrate specificity in prokaryotic and eukaryotic rhomboids, Curr. Biol. 12 (2002) 1507–1512.
[11] S. Urban, M.S. Wolfe, Reconstitution of intramembrane proteolysis in vitro reveals that pure rhomboid is sufficient for catalysis and specificity, Proc. Natl. Acad. Sci. USA 102 (2005) 1883–1888.
[12] M.K. Lemberg, J. Menendez, A. Misik, M. Garcia, C.M. Koth, M. Freeman, Mechanism of intramembrane proteolysis investigated with purified rhomboid proteases, EMBO J. 24 (2005) 464–472.
[13] C.L. Brooks, M.J. Lemieux, Untangling structure–function relationships in the rhomboid family of intramembrane proteases, Biochim. Biophys. Acta 1828 (2013) 2862–2872.
[14] Y. Wang, Y. Zhang, Y. Ha, Crystal structure of a rhomboid family intramembrane protease, Nature 444 (2006) 179–180.
[15] M.K. Lemberg, M. Freeman, Functional and evolutionary implications of enhanced genomic analysis of rhomboid intramembrane proteases, Genome Res. 17 (2007) 1634–1646.
[16] E.J. Greenblatt, J.A. Olzmann, R.R. Kopito, Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant alpha-1 antitrypsin from the endoplasmic reticulum, Nat. Struct. Mol. Biol. 18 (2011) 1147–1152.
[17] E.V. Koonin, K.S. Makarova, I.B. Rogozin, L. Davidovic, M.C. Letellier, L. Pellegrini, The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers, Genome Biol. 4 (2003) R19.
[18] R.D. Finn, J. Mistry, J. Tate, P. Coggill, A. Heger, J.E. Pollington, O.L. Gavin, P. Gunasekaran, G. Ceric, K. Forslund, L. Holm, E.L. Sonnhammer, S.R. Eddy, A. Bateman, The Pfam protein families database, Nucleic Acids Res. 38 (2010) D211–D222.
[19] C. Adrain, M. Freeman, New lives for old: evolution of pseudoenzyme function illustrated by iRhoms, Nat. Rev. Mol. Cell Biol. 13 (2012) 489–498.

[20] R.P. Baker, S. Urban, Architectural and thermodynamic principles underlying intramembrane protease function, Nat. Chem. Biol. 8 (2012) 759–768.
[21] G. von Heijne, Membrane-protein topology, Nat. Rev. Mol. Cell Biol. 7 (2006) 909–918.
[22] J.C. Pascall, K.D. Brown, Characterization of a mammalian cDNA encoding a protein with high sequence similarity to the Drosophila regulatory protein rhomboid, FEBS Lett. 429 (1998) 337–340.
[23] O. Lohi, S. Urban, M. Freeman, Diverse substrate recognition mechanisms for rhomboids; thrombomodulin is cleaved by Mammalian rhomboids, Curr. Biol. 14 (2004) 236–241.
[24] Y. Wang, X. Guan, K.L. Fok, S. Li, X. Zhang, S. Miao, S. Zong, S.S. Koide, H.C. Chan, L. Wang, A novel member of the Rhomboid family, RHBDD1, regulates BIK-mediated apoptosis, Cell. Mol. Life Sci. 65 (2008) 3822–3829.
[25] L. Fleig, N. Bergbold, P. Sahasrabudhe, B. Geiger, L. Kaltak, M.K. Lemberg, Ubiquitin-dependent intramembrane rhomboid protease promotes ERAD of membrane proteins, Mol. Cell 47 (2012) 558–569.
[26] G.A. McQuibban, S. Saurya, M. Freeman, Mitochondrial membrane remodelling regulated by a conserved rhomboid protease, Nature 423 (2003) 537–541.
[27] D.V. Jeyaraju, L. Xu, M.C. Letellier, S. Bandaru, R. Zunino, E.A. Berg, H.M. McBride, L. Pellegrini, Phosphorylation and cleavage of presenilin-associated rhomboid-like protein (PARL) promotes changes in mitochondrial morphology, Proc. Natl. Acad. Sci. USA 103 (2006) 18562–18567.
[28] R. Tsruya, A. Wojtalla, S. Carmon, S. Yogev, A. Reich, E. Bibi, G. Merdes, E. Schejter, B.Z. Shilo, Rhomboid cleaves Star to regulate the levels of secreted Spitz, EMBO J. 26 (2007) 1211–1220.
[29] E. Erez, E. Bibi, Cleavage of a multispanning membrane protein by an intramembrane serine protease, Biochemistry 48 (2009) 12314–12322.
[30] C. Wan, J. Fu, Y. Wang, S. Miao, W. Song, L. Wang, Exosome-related multi-pass transmembrane protein TSAP6 is a target of rhomboid protease RHBDD1-induced proteolysis, PLoS One 7 (2012) e37452.
[31] X. Lei, Y.M. Li, The processing of human rhomboid intramembrane serine protease RHBDL2 is required for its proteolytic activity, J. Mol. Biol. 394 (2009) 815–825.
[32] M.K. Lemberg, Intramembrane proteolysis in regulated protein trafficking, Traffic 12 (2011) 1109–1118.
[33] S. Urban, Taking the plunge: integrating structural, enzymatic and computational insights into a unified model for membrane-immersed rhomboid proteolysis, Biochem. J. 425 (2010) 501–512.
[34] S. Urban, M. Freeman, Substrate specificity of rhomboid intramembrane proteases is governed by helix-breaking residues in the substrate transmembrane domain, Mol. Cell 11 (2003) 1425-1134.
[35] S.M. Moin, S. Urban, Membrane immersion allows rhomboid proteases to achieve specificity by reading transmembrane segment dynamics, Elife 1 (2012) e00173.
[36] Y. Akiyama, S. Maegawa, Sequence features of substrates required for cleavage by GlpG, an *Escherichia coli* rhomboid protease, Mol. Microbiol. 64 (2007) 1028–1037.
[37] K. Strisovsky, H.J. Sharpe, M. Freeman, Sequence-specific intramembrane proteolysis: identification of a recognition motif in rhomboid substrates, Mol. Cell 36 (2009) 1048–1059.
[38] C.T. Esmon, N.L. Esmon, The link between vascular features and thrombosis, Annu. Rev. Physiol. 73 (2011) 503–514.
[39] Y.Y. Hsu, G.Y. Shi, C.H. Kuo, S.L. Liu, C.M. Wu, C.Y. Ma, F.Y. Lin, H.Y. Yang, H.L. Wu, Thrombomodulin is an ezrin-interacting protein that controls epithelial morphology and promotes collective cell migration, FASEB J. 26 (2012) 3440–3452.
[40] D.E. Jackson, T.J. Tetaz, H.H. Salem, C.A. Mitchell, Purification and characterization of two forms of soluble thrombomodulin from human urine, Eur. J. Biochem. 221 (1994) 1079–1087.
[41] T.L. Cheng, Y.T. Wu, H.Y. Lin, F.C. Hsu, S.K. Liu, B.I. Chang, W.S. Chen, C.H. Lai, G.Y. Shi, H.L. Wu, Functions of rhomboid family protease RHBDL2 and thrombomodulin in wound healing, J. Invest. Dermatol. 131 (2011) 2486–2494.
[42] C.S. Shi, G.Y. Shi, Y.S. Chang, H.S. Han, C.H. Kuo, C. Liu, H.C. Huang, Y.J. Chang, P.S. Chen, H.L. Wu, Evidence of human thrombomodulin domain as a novel angiogenic factor, Circulation 111 (2005) 1627–1636.
[43] J.C. Pascall, K.D. Brown, Intramembrane cleavage of ephrinB3 by the human rhomboid family protease, RHBDL2, Biochem. Biophys. Res. Commun. 317 (2004) 244–252.
[44] D. Arvanitis, A. Davy, Eph/ephrin signaling: networks, Genes Dev. 22 (2008) 416–429.
[45] M. Hattori, M. Osterfield, J.G. Flanagan, Regulated cleavage of a contact-mediated axon repellent, Science 289 (2000) 1360–1365.
[46] M. Tanaka, K. Sasaki, R. Kamata, R. Sakai, The C-terminus of ephrin-B1 regulates metalloproteinase secretion and invasion of cancer cells, J. Cell Sci. 120 (2007) 2179–2189.
[47] K.T. Lin, S. Sloniowski, D.W. Ethell, I.M. Ethell, Ephrin-B2-induced cleavage of EphB2 receptor is mediated by matrix metalloproteinases to trigger cell repulsion, J. Biol. Chem. 283 (2008) 28969–28979.
[48] P. Saftig, K. Reiss, The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: novel drug targets with therapeutic potential? Eur. J. Cell Biol. 90 (2011) 527–535.
[49] J.J. Peschon, J.L. Slack, P. Reddy, K.L. Stocking, S.W. Sunnarborg, D.C. Lee, W.E. Russell, B.J. Castner, R.S. Johnson, J.N. Fitzner, R.W. Boyce, N. Nelson, C.J. Kozlosky, M.F. Wolfson, C.T. Rauch, D.P. Cerretti, R.J. Paxton, C.J. March, R.A. Black, An essential role for ectodomain shedding in mammalian development, Science 282 (1998) 1281–1284.
[50] U. Sahin, G. Weskamp, K. Kelly, H.M. Zhou, S. Higashiyama, J. Peschon, D. Hartmann, P. Saftig, C.P. Blobel, Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands, J. Cell Biol. 164 (2004) 769–779.
[51] P.E. Jorgensen, E. Nexo, S.S. Poulsen, M. Almendingen, T. Berg, Processing of epidermal growth factor in the rat submandibular gland by Kallikrein-like enzymes, Growth Factors 11 (1994) 113–123.
[52] C. Adrain, K. Strisovsky, M. Zettl, L. Hu, M.K. Lemberg, M. Freeman, Mammalian EGF receptor activation by the rhomboid protease RHBDL2, EMBO Rep. 12 (2011) 421–427.
[53] H.J. Liao, G. Carpenter, Regulated intramembrane cleavage of the EGF receptor, Traffic 13 (2012) 1106–1112.
[54] K. Haglund, I. Dikic, The role of ubiquitylation in receptor endocytosis and endosomal sorting, J. Cell Sci. 125 (2012) 265–275.
[55] M. Vecchi, G. Carpenter, Constitutive proteolysis of the ErbB-4 receptor tyrosine kinase by a unique, sequential mechanism, J. Cell Biol. 139 (1997) 995–1003.
[56] W. Zhou, G. Carpenter, Heregulin-dependent trafficking and cleavage of ErbB-4, J. Biol. Chem. 275 (2000) 34737–34743.
[57] C.Y. Ni, M.P. Murphy, T.E. Golde, G. Carpenter, Gamma -secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase, Science 294 (2001) 2179–2181.
[58] A. Petri, J. Ahnfelt-Ronne, K.S. Frederiksen, D.G. Edwards, D. Madsen, P. Serup, J. Fleckner, R.S. Heller, The effect of neurogenin3 deficiency on pancreatic gene expression in embryonic mice, J. Mol. Endocrinol. 37 (2006) 301–316.
[59] A. Kumar, J.R. Gibbs, A. Beilina, A. Dillman, R. Kumaran, D. Trabzuni, M. Ryten, R. Walker, C. Smith, B.J. Traynor, J. Hardy, A.B. Singleton, M.R. Cookson, Age-associated changes in gene expression in human brain and isolated neurons, Neurobiol. Aging (2012).
[60] I. Braakman, N.J. Bulleid, Protein folding and modification in the mammalian endoplasmic reticulum, Annu. Rev. Biochem. 80 (2011) 71–99.
[61] R.Y. Hampton, T. Sommer, Finding the will and the way of ERAD substrate retrotranslocation, Curr. Opin. Cell Biol. 24 (2012) 460–466.
[62] M. Zettl, C. Adrain, K. Strisovsky, V. Lastun, M. Freeman, Rhomboid family pseudoproteases use the ER quality control machinery to regulate intercellular signaling, Cell 145 (2011) 79–91.
[63] R.S. Chanthaphavong, P.A. Loughran, T.Y. Lee, M.J. Scott, T.R. Billiar, A Role for cGMP in inducible nitric-oxide synthase (iNOS)-induced tumor necrosis factor (TNF) alpha-converting enzyme (TACE/ADAM17) activation, translocation, and TNF receptor 1 (TNFR1) shedding in hepatocytes, J. Biol. Chem. 287 (2012) 35887–35898.
[64] D.R. McIlwain, P.A. Lang, T. Maretzky, K. Hamada, K. Ohishi, S.K. Maney, T. Berger, A. Murthy, G. Duncan, H.C. Xu, K.S. Lang, D. Haussinger, A. Wakeham, A. Itie-Youten, R. Khokha, P.S. Ohashi, C.P. Blobel, T.W. Mak, iRhom2 regulation of TACE controls TNF-mediated protection against Listeria and responses to LPS, Science 335 (2011) 229–232.
[65] P.M. Wojnarowicz, D.M. Provencher, A.M. Mes-Masson, P.N. Tonin, Chromosome 17q25 genes, RHBDF2 and CYGB, in ovarian cancer, Int. J. Oncol. 40 (2012) 1865–1880.
[66] D.C. Blaydon, S.L. Etheridge, J.M. Risk, H.C. Hennies, L.J. Gay, R. Carroll, V. Plagnol, F.E. McRonald, H.P. Stevens, N.K. Spurr, D.T. Bishop, A. Ellis, J. Jankowski, J.K. Field, I.M. Leigh, A.P. South, D.P. Kelsell, RHBDF2 mutations are associated with tylosis, a familial esophageal cancer syndrome, Am. J. Hum. Genet. 90 (2012) 340–346.
[67] C. Adrain, M. Zettl, Y. Christova, N. Taylor, M. Freeman, Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE, Science 335 (2012) 225–228.
[68] O.M. Siggs, N. Xiao, Y. Wang, H. Shi, W. Tomisato, X. Li, Y. Xia, B. Beutler, iRhom2 is required for the secretion of mouse TNF alpha, Blood 119 (2012) 5769–5771.
[69] J. Schlondorff, J.D. Becherer, C.P. Blobel, Intracellular maturation and localization of the tumour necrosis factor alpha convertase (TACE), Biochem. J. 347 (Pt 1) (2000) 131–138.
[70] P.D. Issuree, T. Maretzky, D.R. McIlwain, S. Monette, X. Qing, P.A. Lang, S.L. Swendeman, K.H. Park-Min, N. Binder, G.D. Kalliolias, A. Yarilina, K. Horiuchi, L.B. Ivashkiv, T.W. Mak, J.E. Salmon, C.P. Blobel, iRHOM2 is a critical pathogenic mediator of inflammatory arthritis, J. Clin. Invest. (2013).
[71] M.K. Lemberg, Sampling the membrane: function of rhomboid-family proteins, Trends Cell Biol. (2013), http://dx.doi.org/10.1016/j.tcb.2013.01.002.
[72] Z. Yan, H. Zou, F. Tian, J.R. Grandis, A.J. Mixson, P.Y. Lu, L.Y. Li, Human rhomboid family-1 gene silencing causes apoptosis or autophagy to epithelial cancer cells and inhibits xenograft tumor growth, Mol. Cancer Ther. 7 (2008) 1355–1364.
[73] H. Zou, S.M. Thomas, Z.W. Yan, J.R. Grandis, A. Vogt, L.Y. Li, Human rhomboid family-1 gene RHBDF1 participates in GPCR-mediated transactivation of EGFR growth signals in head and neck squamous cancer cells, FASEB J. 23 (2009) 425–432.
[74] S. Saarinen, P. Vahteristo, R. Lehtonen, K. Aittomaki, V. Launonen, T. Kiviluoto, L.A. Aaltonen, Analysis of a Finnish family confirms RHBDF2 mutations as the underlying factor in tylosis with esophageal cancer, Fam. Cancer 11 (2012) 525–528.
[75] T. Nakagawa, A. Guichard, C.P. Castro, Y. Xiao, M. Rizen, H.Z. Zhang, D. Hu, A. Bang, J. Helms, E. Bier, R. Derynck, Characterization of a human rhomboid homolog, p100hRho/RHBDF1, which interacts with TGF-alpha family ligands, Dev. Dyn. 233 (2005) 1315–1331.
[76] M. Knop, A. Finger, T. Braun, K. Hellmuth, D.H. Wolf, Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast, EMBO J. 15 (1996) 753–763.
[77] J. Bordallo, R.K. Plemper, A. Finger, D.H. Wolf, Der3p/Hrd1p is required for endoplasmic reticulum-associated degradation of misfolded lumenal and integral membrane proteins, Mol. Biol. Cell 9 (1998) 209–222.
[78] P. Carvalho, V. Goder, T.A. Rapoport, Distinct ubiquitin–ligase complexes define convergent pathways for the degradation of ER proteins, Cell 126 (2006) 361–373.
[79] B.N. Lilley, H.L. Ploegh, A membrane protein required for dislocation of misfolded proteins from the ER, Nature 429 (2004) 834–840.
[80] Y. Ye, Y. Shibata, C. Yun, D. Ron, T.A. Rapoport, A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol, Nature 429 (2004) 841–847.

[81] J.M. Younger, L. Chen, H.Y. Ren, M.F. Rosser, E.L. Turnbull, C.Y. Fan, C. Patterson, D.M. Cyr, Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator, Cell 126 (2006) 571–582.
[82] J.C. Christianson, J.A. Olzmann, T.A. Shaler, M.E. Sowa, E.J. Bennett, C.M. Richter, R.E. Tyler, E.J. Greenblatt, J. Wade Harper, R.R. Kopito, Defining human ERAD networks through an integrative mapping strategy, Nat. Cell Biol. 14 (2011) 93–105.
[83] B. Wang, H. Heath-Engel, D. Zhang, N. Nguyen, D.Y. Thomas, J.W. Hanrahan, G.C. Shore, BAP31 interacts with Sec61 translocons and promotes retrotranslocation of CFTRDeltaF508 via the derlin-1 complex, Cell 133 (2008) 1080–1092.
[84] Y. Oda, T. Okada, H. Yoshida, R.J. Kaufman, K. Nagata, K. Mori, Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation, J. Cell Biol. 172 (2006) 383–393.
[85] J.A. Olzmann, C.M. Richter, R.R. Kopito, Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover, Proc. Natl. Acad. Sci. USA 110 (2013) 1345–1350.
[86] N.B. Ahmedli, Y. Gribanova, C.C. Njoku, A. Naidu, A. Young, E. Mendoza, C.K. Yamashita, R.K. Ozgul, J.E. Johnson, D.A. Fox, D.B. Farber, Dynamics of the rhomboid-like Rhbdd2 expression in mouse retina and involvement of its human ortholog in retinitis pigmentosa, J. Biol. Chem. (2013).
[87] M.C. Abba, H. Sun, K.A. Hawkins, J.A. Drake, Y. Hu, M.I. Nunez, S. Gaddis, T. Shi, S. Horvath, A. Sahin, C.M. Aldaz, Breast cancer molecular signatures as determined by SAGE: correlation with lymph node status, Mol. Cancer Res. 5 (2007) 881–890.
[88] M.C. Abba, E. Lacunza, M.I. Nunez, A. Colussi, M. Isla-Larrain, A. Segal-Eiras, M.V. Croce, C.M. Aldaz, Rhomboid domain containing 2 (RHBDD2): a novel cancer-related gene over-expressed in breast cancer, Biochim. Biophys. Acta 1792 (2009) 988–997.
[89] E. Lacunza, R. Canzoneri, M.E. Rabassa, A. Zwenger, A. Segal-Eiras, M.V. Croce, M.C. Abba, RHBDD2: a 5-fluorouracil responsive gene overexpressed in the advanced stages of colorectal cancer, Tumour Biol. 33 (2012) 2393–2399.
[90] O.A. Pierrat, K. Strisovsky, Y. Christova, J. Large, K. Ansell, N. Bouloc, E. Smiljanic, M. Freeman, Monocyclic beta-lactams are selective, mechanism-based inhibitors of rhomboid intramembrane proteases, ACS Chem. Biol. 6 (2011) 325–335.
[91] S. Cipolat, T. Rudka, D. Hartmann, V. Costa, L. Serneels, K. Craessaerts, K. Metzger, C. Frezza, W. Annaert, L. D'Adamio, C. Derks, T. Dejaegere, L. Pellegrini, R. D'Hooge, L. Scorrano, B. De Strooper, Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling, Cell 126 (2006) 163–175.
[92] J.R. Chao, E. Parganas, K. Boyd, C.Y. Hong, J.T. Opferman, J.N. Ihle, Hax1-mediated processing of HtrA2 by Parl allows survival of lymphocytes and neurons, Nature 452 (2008) 98–102.
[93] S.M. Jin, M. Lazarou, C. Wang, L.A. Kane, D.P. Narendra, R.J. Youle, Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL, J. Cell Biol. 191 (2010) 933–942.
[94] E. Deas, H. Plun-Favreau, S. Gandhi, H. Desmond, S. Kjaer, S.H. Loh, A.E. Renton, R.J. Harvey, A.J. Whitworth, L.M. Martins, A.Y. Abramov, N.W. Wood, PINK1 cleavage at position A103 by the mitochondrial protease PARL, Hum. Mol. Genet. 20 (2011) 867–879.
[95] G. Shi, J.R. Lee, D.A. Grimes, L. Racacho, D. Ye, H. Yang, O.A. Ross, M. Farrer, G.A. McQuibban, D.E. Bulman, Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease, Hum. Mol. Genet. 20 (2011) 1966–1974.
[96] C. Meissner, H. Lorenz, A. Weihofen, D.J. Selkoe, M.K. Lemberg, The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking, J. Neurochem. 117 (2011) 856–867.
[97] S. Sekine, Y. Kanamaru, M. Koike, A. Nishihara, M. Okada, H. Kinoshita, M. Kamiyama, J. Maruyama, Y. Uchiyama, N. Ishihara, K. Takeda, H. Ichijo, Rhomboid protease PARL mediates the mitochondrial membrane potential loss-induced cleavage of PGAM5, J. Biol. Chem. 287 (2012) 34635–34645.