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The role of rhomboid superfamily members in protein homeostasis: Mechanistic insight and physiological implications

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Abstract

Cells are equipped with protein quality control pathways in order to maintain a healthy proteome; a process known as protein homeostasis. Dysfunction in protein homeostasis leads to the development of many diseases that are associated with proteinopathies. Recently, the rhomboid superfamily has attracted much attention concerning their involvement in protein homeostasis. While their functional role has become much clearer in the last few years, their systemic significance in mammals remains elusive. Here we delineate the current knowledge of rhomboids in protein quality control and how these functions are integrated at the organismal level.

Keywords

rhomboid protease; rhomboid pseudoprotease; derlins; protein homeostasis; ERAD

1. Introduction

Proteins serve as the primary workhorses for executing a vast majority of cellular and organismal functions. Unfortunately, misfolding of proteins is a common occurrence, either due to chemical and UV damage, imbalanced subunit synthesis, or genetic mutation [1–3]. Unchecked accumulation of these aberrant proteins generates constant cellular stress and underlies many of the most pressing human maladies, including aging, cancer and neurodegenerative diseases [2,4,5]. To offset the catastrophic effect of unwanted proteins, organisms are equipped with quality control systems that are vital for surveillance, prevention, and rescue of protein defects [6–9].

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Declaration of competing interest

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Recent advances have shown that the rhomboid superfamily are involved in multiple facets of protein homeostasis [10–12]. In general, the rhomboid protein family carries out many membrane-related processes such as development, signaling, parasitic invasion, and protein trafficking (reviewed in [13]). A prominent feature of rhomboids is their ability to cleave their membrane-anchored substrates at specific sites within the lipid bilayer; a process mediated by rhomboid proteases through their conserved serine-histidine dyad at the active site [14–18]. A subclass of rhomboids has evolved from their rhomboid protease predecessors that are not proteases; they lack catalytic residues for proteolysis and are known as rhomboid pseudoproteases. Despite the absence of protease activity, rhomboid pseudoproteases carry out similar biological processes as their rhomboid protease counterparts and function in lipid homeostasis, protein trafficking, sterol regulation, and signaling [11,19]. Although growing evidence suggests that both rhomboid proteases and pseudoproteases have central roles in safeguarding the proteome, little data exist regarding their systemic significance in mammals. In this review, we will discuss the mechanistic underpinnings of rhomboids in the context of protein quality control and describe current knowledge on their role in maintaining a healthy proteome in both health and disease. Overall, understanding rhomboid function within a broader organismal perspective will reveal their importance as therapeutic targets in many diseases.

2. Rhomboid pseudoproteases

2.1 Derlins

Derlins were first discovered as key mediators of ER (Endoplasmic Reticulum) protein quality control in yeast and mammals (described in Section 2.1.1) [20–23]. Based on sequence and structural homology, derlins share structural similarities to the rhomboid-like superfamily [24]. Specifically, they are ER-resident integral membrane proteins and have been predicted to span the lipid bilayer 6 times [24]. The current knowledge and thinking of this subclass of the rhomboid family will be discussed below.

2.1.1 The unDERLying role in ERAD and retrotranslocation—Maintaining proteostasis is particularly challenging in the ER where the high demand for protein synthesis generates constant misfolding stress [9]. To off-set the catastrophic effects that accompany defective protein accumulation, misfolded ER proteins are targeted for degradation via ER-associated degradation (ERAD) [25,26]. The span of substrates for ERAD is quite large; ranging from ER-localized misassembled proteins to misfolded membrane and luminal proteins [27–30]. During ERAD, substrates destined for degradation are tagged with ubiquitin by an E3 ligase, delivered back into the cytoplasm by a dedicated export machinery and then degraded by the cytosolic 26S proteasome [20]. Perhaps one of the most intriguing features of ERAD is the requirement of removing substrates from their ER-resident to their final destination in the cytosol for proteasomal degradation; a process known as retrotranslocation [31] which is powered by Cdc48/p97 AAA-ATPase [32–34].

Retrotranslocation requires a route or channel for the removal of misfolded proteins through or from the ER membrane. The identification of an exit channel(s) has been a challenging problem that is only now yielding answers [35–40]. Derlins have emerged as likely

candidates for transporting ERAD substrates out of the ER. For instance, human Derlin-1 was initially discovered by two independent groups for its role in assisting a viral component, US11, in degrading class 1 MHC heavy chain (MHC-1) within the infected host [22,41]. Derlin-1 is an ER-resident multi-spanning protein with homology to the yeast Der1, which is involved in ERAD [42]. Although many studies demonstrated that derlins assist in ERAD of several substrates[43–48], their direct function in retrotranslocation remained obscure. Previous *in vitro* and structural studies by Rapoport and colleagues suggested that the multi-spanning yeast E3 ligase Hrd1 serves as a channel for luminal substrates [37–39]. An analogous channel for ERAD membrane substrates remained to be determined until Neal and colleagues improved the understanding of membrane substrate retrotranslocation by screening a complete collection of yeast mutants via SPOCK (single plate orf compendium kit), which consists of 5,808 yeast strain array of non-essential gene deletion mutants and essential DAmP gene mutants [49] and identified yeast derlin Dfm1 as an independent, dedicated and specific mediator for the retrotranslocation of many ERAD membrane substrates (Fig. 1) [36]. Furthermore, both human Derlin-1 and yeast Dfm1 contain a unique C-terminal SHP box for direct recruitment of Cdc48/p97 AAA-ATPase to the ER membrane and this interaction is essential for removing ERAD substrates [36,40]. This finding contradicted previous results in which Dfm1 had no role in ERAD [47,50]. This was due to *dfm1*Δ-nulls being rapidly suppressed; masking the effect of *dfm1*Δ on retrotranslocation [36]. Accordingly, the fast curation of the SPOCK screen has revealed Dfm1 as being one of the major mediators of ERAD.

Sequence conservation and structural homology suggest that derlins share similarities with rhomboid-like superfamily [51]. The structures of *E. coli* and *H. influenzae* rhomboid protease GlpG [18,52,53], and a body of structure-function analyses, molecular modeling and mechanistic studies on the rhomboid superfamily from over a decade have elucidated some of the mechanistic principles that may be at play in derlin-mediated retrotranslocation (see recent review in [54]). Although the structure of the bacterial rhomboid complexed with substrate is lacking, biochemical studies show that rhomboid proteases not only recognize structurally unstable single transmembrane domains [55–57], but they also recognize regions of extramembrane domains [58] and some features within polytopic transmembrane proteins [51,59,60]. Furthermore, function of rhomboid proteins as surveyors of the membrane may be aided by their unusually fast diffusion in the membrane for substrate targeting [61], possibly aided by their compact fold and small hydrophobic thickness that may induce local deformation of the lipid bilayer [15,53].

Despite the absence of protease activity, derlins have retained conserved rhomboid residues [36,40]. This conservation implies the intriguing idea that derlins have retained the biological properties of rhomboids for use in retrotranslocation. In support of this idea, we and others previously published that human and yeast derlins utilize their conserved rhomboid motifs for removing misfolded substrates from the ER [24,36]. A popular working hypothesis is that derlins have retained membrane perturbing properties of its bacterial counterpart, GlpG, to facilitate the movement of substrates across the membrane [36,62]. Recent work from the Rapoport lab has shown that a yeast derlin and Dfm1 paralog, Der1, possesses membrane perturbation properties to assist in the retrotranslocation of ER luminal substrates. [63]. Using cryo-electron microscopy, the authors determined the structure of the

Hrd1 complex, which is comprised of monomers of Hrd1, Der1, Hrd3, Yos9, and Usa1. While it had previously been reported that a Hrd1 dimer is the retrotranslocon for soluble proteins, the revised structure establishes that monomeric Hrd1 acts as a half channel and interacts with Der1, which forms the other half of the channel [38,63]. As shown by molecular dynamic (MD) simulations, both Der1 and Hrd1 can induce distortion of the lipid bilayer. Der1 contains a lateral gate between TM2 and TM5 and TM2 contains hydrophilic residues that MD simulations predict cause lipid thinning that is proposed to aid in the retrotranslocation of substrates. Indeed, mutation of these residues to hydrophobic residues slows the degradation rate of luminal ERAD clients. This important discovery supports the hypothesis that the membrane perturbation ability of rhomboids can aid in retrotranslocation of proteins.

2.1.2 Derlins strike first in the pre-emptive QC Pathway—The ER employs various proteostatic strategies for maintaining a healthy proteome. Most notable is the ER's ability to launch a pre-emptive strike on a selection of proteins prior to their infiltration in the ER. Put less poetically, the ER's main line of defense is to prevent protein overload. This preventative system is known as the ER stress-induced pre-emptive quality control pathway (ERpQC) [64,65]. In this system, newly synthesized polypeptides are co-translationally inserted into the entry gate of the ER, which is the Sec61 channel. Studies by Noshito and colleages have shown that during acute ER stress, derlins are recruited to the Sec61 translocon pore where they reroute ER-targeted substrates to an E3 ligase to initiate ubiquitin tagging and degradation of the substrates by the cytosolic 26S proteasome [64,65]. This study implies that derlins function at the nexus of the mechanistically distinct pathways of ERpQC and ERAD. Exactly how derlins capture incoming substrate and the extent to which rhomboid features are employed during the derlin-backed rerouting step in ERpQC remains as an open question.

2.1.3 Physiological role of derlins—The physiological role of derlin-mediated ERAD has been difficult to study due to the embryonic and perinatal lethality of mice deficient for derlin homologs: Derlin-1 and Derlin-2, respectively (Table 1) [66,67]. The recent generation of cell-type-specific derlin-deficient mice has offered a unique opportunity to delineate the significance of derlins in physiology (Table 1). Schwann-cell specific Derlin-2 KO mice results in late onset of neuropathy with myelin exhibiting severe defects in its morphology and function [68]. This defect is most likely the result of abnormal maintenance of myelin protein in the ER and consequent disruption in Schwann cellular function. Furthermore, Ren and colleagues investigated the underlying functional role of Derlin-2 in kidney-derived podocyte cells [69]. Harsh environmental conditions experienced by podocytes and renal protein mutations contribute to protein misfolding in the ER of podocytes, evoking constant ER stress [70]. Furthermore, ER stress causes an onset of many kidney diseases such as diabetic nephropathy, renal fibrosis, and ischemia-reperfusion [71]. It is proposed that podocytes utilize Derlin-2 as a protein quality control mechanism in order to cope with persistent ER stress [69,72]. Patients with diabetic nephropathy and corresponding kidney disease mice models have upregulated Derlin-2 levels [69]. Furthermore, tissue culture studies demonstrate that Derlin-2 overexpression is positively correlated with the survival of ER-stressed podocytes [69]. Overall, both studies demonstrate

that Derlin-2 deficient Schwann cells and podocytes are functionally compromised when the burden of misfolded substrates becomes insurmountable.

This collection of results suggest derlins play a prominent role in safeguarding the proteome in normal physiology. If this is the case, tissues with high secretory demand should be severely affected when derlin function is compromised. Contrary to this expectation, developing Schwann, hepatocytes, podocytes and B-cells are able to cope with derlin deficiency and ER stress under normal basal conditions [66–69]. This could be due to cellular adaptation from compensatory pathways such as functional redundancy amongst all three derlin paralogs, autophagy, or alternative ER protein quality control pathways. How cells handle the accumulation of certain substrates should be considered on a case-by-case scenario for different cell types.

Along with protecting the proteome, derlins can control abundance of specific substrates, which would modulate the activity of the substrates within the cell. For example, tissue culture studies have shown that derlins degrade a wide range of substrates including potassium channels (K_{ATP}), ENaC, ApoB, cystic fibrosis transmembrane conductance regulator (CFTR), to name a few [40,73–75]. This suggest derlins can regulate basic physiological processes in a substrate-specific manner. Ongoing effort of generating derlin-deficient animal models will bode well for researchers seeking to understand the physiological role of derlins. It is possible that the three mammalian derlin homologs exhibit functional redundancy. Thus, future works in utilizing tissue-specific double or triple derlin knockout animal models would address this potential problem.

2.1.4 Disease role of derlins—Cancer cells are highly proliferative in nutrient-deprived and hypoxic conditions, making them prone to protein misfolding throughout the cell [70]. Cancer cells are susceptible to protein misfolding stress within the ER where the demand of protein folding is high [76]. The ability of cancer cells to cope with ER stress leads to their survival and chemo-resistance [76]. Several studies have shown that cancer cells cope with ER stress by activating ERAD. For example, derlin mRNA is overexpressed in breast cancer (Derlin-1 and Derlin-3) and colon cancer (Derlin-1) in order to mitigate ER stress (Table 2) [77–81]. In contrast, another study has shown that colon cancer cells can downregulate Derlin-3 through hypermethylation of its promoter region in order to promote cancer cell survial. For example, cancer cells have an enormous demand for ATP to fuel their growth, and glycolysis, as opposed to oxidative phosphorylation, is better suited to meet this demand [82]. This metabolic switch to glycolysis is accompanied by enhancement of glucose uptake through stabilization of glucose transporter, GLUT1, levels [83]. This GLUT1 stabilization is a result of transcriptional inactivation of Derlin-3, which is normally responsible for targeting the transporter for degradation [83]. Ultimately, stabilized GLUT1 leads to increased uptake in glucose which supports the high energy demand of a proliferative cancer cell. Altogether, the above studies suggest that derlin upregulation and downregulation in different cancer cell types can support cancer cell survival and metabolism, respectively. Understanding the basic biological function of derlins during cancer progression warrants future investigation and would serve as a window into developing derlins as a therapeutic target or new biomarker for early diagnosis for cancer.

2.2 Dsc2 and UBAC2

Structural homology demonstrates both yeast Dsc2 and mammalian UBA Domain Containing 2 (UBAC2) belong to the rhomboid pseudoprotease class [40,84,85]. Both proteins have significantly diverged in their primary sequence, with 20% similarity between the two proteins [85]. Furthermore, both Dsc2 and UBAC2 contain a C-terminal motif known as the ubiquitin-associated (UBA) domain that directly interacts with ubiquitin [85,86]. The current knowledge on their biological function will be discussed in detail below.

2.2.1 Dsc2: A lever for cholesterol and sphingolipid homeostasis—Cell cholesterol is under constant multi-layered control. This is mainly regulated by an ER resident transcription factor, Sterol regulatory element-binding protein (SREBP), which is responsible for transcribing genes involved in sterol synthesis, low-density lipoprotein (LDL) receptor, and other lipid-related proteins [87,88]. SREBP itself is also controlled by feedback regulation to which the overarching concept is simple. When cellular cholesterol is low, SREBP is activated by sequential cleavage by Golgi-resident Site-1 and Site-2 proteases, allowing SREBP-mediated sterol synthesis to occur. When cholesterol is high, SREBP is inactive, followed by less sterol synthesis [89]. Notably, the SREBP pathway is conserved in *S. pombe,* with the exception that *S. pombe* does not have Site-1 and Site-2 proteases. The detailed knowledge of SREBP regulation in fission yeast comes from an ongoing odyssey of inquiry by the Espenshade laboratory leading to a collection of basic insights [85,90–92]. Stewart and colleagues utilized a genetic selection screen and discovered Dsc E3 ubiquitin ligase complex is required for the cleavage of fission yeast SREBP, Sre1 [92]. This E3 ligase complex is localized in the ER and Golgi membrane and is comprised of E3 ligase Dsc1, Dsc2, Dsc3 and Dsc4 [85]. Most noteworthy, Dsc2 is homologous to rhomboid pseudoproteases [85]. All rhomboid proteins characterized to date specifically binds their substrates in the plane of the membrane [12]. Based on Dsc2's connection with the rhomboid superfamily, this leads to the idea that Dsc2 is an integral participant in SREBP recognition and binding. Furthermore, recent studies by Teis' laboratory has shown that in baker's yeast, the Dsc E3 ubiquitin ligase complex, which contains the rhomboid pseudoprotease Dsc2, targets a negative regulator of sphingolipid biosynthesis pathway, Orm2, for degradation in the endosome and Golgi apparatus; a pathway known as Endosome Golgi-Associated Degradation (EGAD) [93]. This pioneering study places Dsc2 at the heart of sphingolipid homeostasis- a lipid that is critical for a plethora of cell biological processes, including growth, apoptosis, cell migration and inflammatory responses [94].

Several biochemical studies have shed light on the mechanism for rhomboid pseudoprotease Dsc2 function. For example, Dsc2 plays an important structural role in linking other members of the Dsc E3 ligase complex together [85]. In addition, Dsc2 is able to bind to ubiquitin *in vitro*, which is mediated by its UBA domain located at the C-terminus [85]. However, Lloyd et al., showed that the UBA domain is dispensable for cleavage of fission yeast SREBP, Sre1[85]. Additional studies are needed to precisely understand how ubiquitin binding contributes to Dsc2 function. Overall, these biochemical studies on Dsc2 function have opened the door to a number of questions: What is the physiological role of Dsc2? To

what extent are Dsc2's rhomboid features utilized in SREBP activation or Orm2 degradation? Just like its derlin counterpart, is Dsc2 directly involved in extracting Orm2 and other membrane substrates from the endosome or Golgi membrane? These questions will be interesting avenues to explore in the next few years.

2.2.2 UBAC2 role in energy homeostasis—A BLASTP search revealed that mammalian UBAC2 has significant similarity to yeast Dsc2 [85]. Just like Dsc2, UBAC2 also has a C-terminal UBA domain, which is predicted to bind to ubiquitin. Indeed, the purified recombinant UBA tail of UBAC2 is able to bind to polyubiquitin chains, suggesting involvement of UBAC2 in the ubiquitin-proteasome protein degradation pathway [86]. Consistent with this idea, UBAC2 knockdown led to stabilization of mutant alpha1-antitrypsin, a well-known ERAD substrate [86]. Furthermore, a previous study has shown that UBAC2 contributes to energy homeostasis in mammals in a manner that is distinct from its proposed role in ERAD as described above. UBAC2 was shown to specifically restrict trafficking of UBXD8 from the ER to lipid droplets (LDs) where it is known to regulate the rate limiting enzyme in lipid hydrolysis [95]. Hence, UBAC2 strongly contributes to energy homeostasis by controlling cellular fat storage. It will be interesting to understand how UBAC2 mediated retention of UBXD8 in the ER is regulated.

2.2.3 Disease role of UBAC2—Genome-wide associate studies (GWAS) have strongly linked single-nucleotide polymorphisms (SNPs) of UBAC2 to Behçet disease (BD) (Table 2) [96]. BD is an inflammatory disease associated with development of lesions throughout the body, particularly the central nervous system [96]. Yamazoe et al, have shown that UBAC2 polymorphisms are elevated in BD [96]. Whether an elevated level of UBAC2 in BD increases the risk for BD remains to be determined. Furthermore, BD pathology is associated with other genes related to ubiquitin-related functions including ubiquitin associated and SH3 domain containing B (*UBASH3B*), small ubiquitin-like modifier 4 (*SUMO4*), and ubiquitin-conjugating enzyme E2Q family-like 1 (*UBE2QL*1) [96]. This suggests that the ubiquitin and protein degradation pathways may contribute to the development of BD. Future studies are warranted to confirm the general validity of these findings and to clarify the underlying mechanism of UBAC2 for this association. In this case, support from animal models will be paramount in establishing UBAC2-mediated causality in BD.

2.3 iRhoms

iRhoms are pseudoproteases that are evolutionarily distinct from derlins and are more closely related to rhomboid proteases. iRhoms are ER-resident integral membrane proteins with seven transmembrane helices. *Drosophila* have one iRhom while humans and mice have two, iRhom1 and iRhom2. iRhoms have diverse roles in cellular function (reviewed in [97]). In this review, we will focus on their role in protein stability and quality control.

2.3.1 iRhoms in regulated protein degradation—iRhoms have a broad role in controlling protein abundance via ERAD, as well as by other mechanisms. The first instance of iRhoms playing a role in protein degradation was shown by the Freeman group [98]. In *Drosophila*, iRhom is exclusively expressed in neurons and knockout flies exhibit long

periods of time in a “sleep-like state”. Because the same sleep-like behavior occurs when epidermal growth factor receptor (EGFR) signaling increases, it was hypothesized that iRhom functions as a negative regulator of EGFR signaling. Indeed, the “sleep-like” phenotype is rescued by neuron-specific expression of iRhom, and, in mammalian cells, iRhoms facilitate the degradation of EGFR ligands. In human cell culture, overexpressed iRhom1 has been demonstrated to cause increased proteasome activity [99]. This increase in proteasome activity was also seen with overexpression of the active rhomboids RHBDL1 and RHBDL2, and their catalytically inactive mutants, but this was not further explored. This hyperactivity was proposed to be mediated through stabilization of proteasome chaperones Pac1 and Pac2 by iRhom1, although the exact mechanism has not been determined. The authors also showed iRhom1 protein levels are elevated under ER stress, indicating that iRhom1 may promote faster degradation of accumulating substrates during ER stress through increasing proteasome activity. iRhom1 is also involved in stabilizing the α subunit of the transcription factor hypoxia inducible factor-1 (Hif1α) by reducing its degradation by the proteasome [100]. Stabilization of Hif1α in hypoxic conditions allows formation of the active transcription factor, which promotes cellular adaptation to hypoxia. Under normal conditions, Rack1 interacts with Hif1α, resulting in Hif1α being degraded by the proteasome. Experimental evidence indicates iRhom1 can bind Rack1, thereby preventing its interaction with Hif1α and leading to stabilization of Hif1α. iRhom1 was also upregulated in breast cancer patients with escalated disease progression, and perhaps this allows cancer cells to adapt to hypoxia through increased Hif1α activity.

3. Rhomboid Proteases

3.1 RHBDL4

Rhomboid proteases were first discovered in Drosophila with an important role in cleaving Spitz, a membrane-bound ligand for the epidermal growth factor receptor (EGFR) [101]. In mammals, there are four rhomboid proteases in the secretory pathway (RHBDL1–4) and one in the mitochondria (PARL). While many rhomboid proteases either have not been well described or are involved in cell signaling, the main described role of mammalian rhomboid RHBDL4 (also known by the gene name Rhbdd1) is in protein quality control.

3.1.1 RHBDL4 in ERAD—RHBDL4 is an ER-resident rhomboid protease that is involved in ERAD (see review [102] and brief discussion in Section 2.1.1). In contrast to derlins, which are primarily involved in retrotranslocating full-length defective proteins from the ER, RHBDL4 cleaves specific membrane substrates into fragments which are then retrotranslocated into the cytoplasm and degraded by the proteasome [51].

Two features of RHBDL4 that are important for its role in ERAD are its ubiquitin interacting motif (UIM) and its Valosin-binding motif (VBM), which recruits the AAA-ATPase p97 [51,103]. The UIM motif on RHBDL4 indicates that substrate recognition and eventual cleavage is mediated by substrate ubiquitination. The VBM motif, which recruits p97 to RHBDL4, is highly conserved across eukaryotes, indicating an evolutionary conserved function for RHBDL4 in ERAD [103].

The Lemberg group was the first to show a role for RHBDL4 in cleaving membrane proteins and targeting them for ERAD [51]. In this seminal work, RHBDL4 was shown to cleave several membrane proteins both in their ectodomains as well as in the membrane spanning segments prior to retrotranslocation and degradation. More recent work from the Lemberg group also identified RHBDL4 as having a role in mediating the turnover of misfolded luminal proteins [104]. RHBDL4 forms a complex with the ERAD components Erlin1 and Erlin2, which function as substrate adaptors for its targeting of luminal proteins. RHBDL4 acts in an alternative ERAD pathway for luminal aggregation-prone proteins, whereby they are first cleaved into fragmets and subsequently removed and degraded by the proteasome, instead of being retrotranslocated in their full-length form.

3.1.2 Physiological Role of RHBDL4—Mammalian cell culture studies have elucidated that RHBDL4 can cleave a wide variety of ERAD substrates. The first identified substrate of RHBDL4 was the α subunit of the pre-T cell receptor (pTα) [51]. While there is not a strict sequence specific degron requirement, it was hypothesized that two basic amino acids in the transmembrane span of pTα triggered its degradation by RHBDL4. In support of this hypothesis, a disease variant of myelin protein zero (MPZ) that contains two basic residues in its transmembrane domain can also be cleaved by RHBDL4 and subsequently degraded. Interestingly, when the pTα degron was introduced into opsin, a multipass membrane protein, it was also cleaved by RHBDL4 [51]. This work established that RHBDL4 has loose sequence requirements for recognition and can cleave both single-pass and multi-pass membrane proteins.

The Munter group exhibited that RHBDL4 is capable of cleaving the ectodomain of amyloid precursor protein (APP) [105]. Cleavage of APP by other proteases can result in amyloid β (Aβ) peptides, which are implicated in Alzheimer's disease. Secretion of Aβ peptides was reduced in cells expressing active RHBDL4 compared to cells with inactive RHBDL4 [105]. However, it is unclear whether RHBDL4 mediated cleavage of APP results in degradation through ERAD. RHBDL4-mediated processing of APP can be influenced by binding of cholesterol to specific motifs in the transmembrane domain of RHBDL4 [106]. Decreased levels of cellular cholesterol resulted in an increase in RHBDL4 mediated APP fragments, suggesting that RHBDL4 activity is influenced by the surrounding lipid environment.

Like other ERAD components, RHBDL4 transcription is increased in response to ER stress [51]. Additionally, expression of catalytically inactive RHBDL4 causes substrate trapping, which induces ER stress [51]. Recent work from the Lemberg group used proteomics to identify ERAD targets of RHBDL4 [107]. By using stable isotopic labeling in cell culture (SILAC) with wildtype RHBDL4 and RHBDL4 mutants, the authors were able to identify possible RHBDL4 substrates. Several of the proteins identified were part of the oligosacharyltransferase (OST) complex, which is responsible for glycosylating newly synthesized proteins [107]. By cleaving these subunits and targeting them for ERAD, RHBDL4 can fine tune glycosylation in the cell, which may mitigate ER stress. RHBDL4 also increases degradation of OST subunits when one component in depleted or increased.

Based on the RHBDL4 substrates identified so far, the range of proteins is very broad without universal structural requirements for cleavage. Past studies have relied on the

overexpression of either RHBDL4 or its substrates; making it difficult to determine which RHBDL4 substrates are physiologically relevant. Outstanding questions which remain in terms of RHBDL4 substrates are (i) what features of a target protein determine whether it is targeted by RHBDL4 and (ii) what is the importance of ubiquitination in targeting substrates.

3.1.3 Disease role of RHBDL4—RHBDL4 is upregulated in both colorectal cancer and glioblastoma (Table 2) [108–111]. However, it is not established whether this has any relation to its role in ERAD, or whether it is due solely to other functions of RHBDL4 in cell signaling pathways. Recently, the Lemberg group explored the role of RHBDL4 in regulating OST complex subunit degradation. They proposed that the upregulation of RHBDL4 seen in several cancer types could be to increase the degradation of excess OST complex subunits that could result from aneuploidy in cancer cells [107]. Alternatively, the involvement of RHBDL4 in cancer could be due to its ability to cleave the proapoptotic protein BIK [112]. The overexpression of RHBDL4 seen in some cancer cells could reduce apoptosis, thereby promoting cancer progression and proliferation. To date, the work associating RHBDL4 with cancer has been done using patient samples and cell culture. To better delineate the role of RHBDL4 in disease, it will be important to establish animal models to study how changes in RHBDL4 expression alter cancer progression.

3.2 YqgP

The close mechanistic relationship between rhomboid proteases and pseudoproteases is highlighted by recent research from the Strisovsky group. This research showed that the bacterial rhomboid YqgP in *Bacillus subtilis* regulates magnesium homeostasis by acting as a protease while also displaying functions similar to derlins [113]. YqgP cleaves the magnesium transporter MgtE under environmental conditions of low magnesium and high manganese or zinc. YqgP has an additional function as a substrate adaptor for FtsH, an ATP dependent protease that works in conjunction with YqgP to degrade MgtE. Importantly, the active site of YqgP, but not its catalytic ability, is required for its interaction with FtsH and cleavage of MgtE by FtsH. This pathway has striking similarities to ERAD, with YqgP playing a similar role to derlins in recruiting other machinery for substrate degradation, while also having a direct role in degrading its substrates. This paper establishes a physiologically important process in bacteria that is regulated by a rhomboid protease, and is an example of protein homeostasis being altered in response to environmental conditions to alleviate cellular stress.

4. Conclusions and Perspectives

In just the past 5 years, we have learned a great deal about the rhomboid superfamily and their importance in protein homeostasis. The rhomboid superfamily is widespread and highly conserved, and it is remarkable to see how fundamental cell biological studies have paved the way to our current knowledge of rhomboid biology in health and diseases. The fundamental knowledge gained regarding the rhomboid protein family’s systemic significance in animal models bodes well in providing a mechanistic and conceptual platform for understanding the broader functions of the rhomboid superfamily. Thus far,

studies on cell-specific rhomboid pseudoprotease knockout mice have made a major contribution towards understanding their physiological role of rhomboid pseudoproteases. The lack of function of some rhomboid pseudoproteases has been demonstrated to activate ER stress responses in cells with large secretory demand. Moreover, biochemical studies in mammalian cell culture show that the rhomboid superfamily target a plethora of substrates and may affect downstream pathways in a substrate-specific manner. Future studies in rhomboid-deficient animal knockouts will shed light on their biological significance. We predict that many fundamental questions about rhomboids under normal and pathophysiological conditions will be addressed in the next few years, including their specific biological role in cancer cells. Accordingly, these studies will provide fundamental knowledge in exploiting the rhomboid superfamily for potential therapeutics and will be an incredibly exciting area of research in the years to come.

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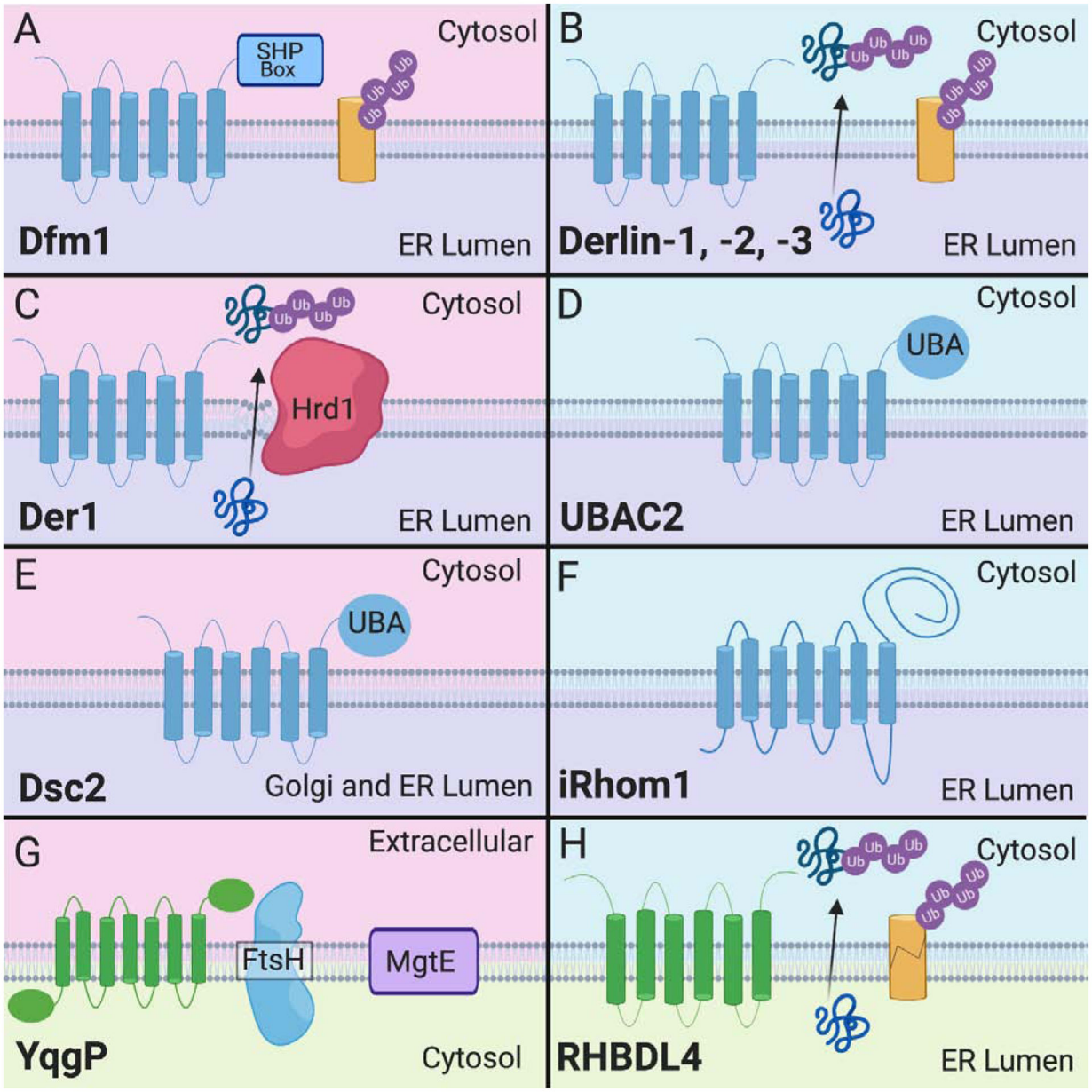

Figure 1. Cellular localization of rhomboids

A-F depicts rhomboid pseudoproteases and G-H depicts rhomboid proteases. Rhomboids depicted in A and C are found in *S. cerevisiae*, E is found in *S. pombe*, G is found in *B. subtilus,* and B, D, F, and H are found in mammals.

Table 1.

Rhomboid member knockout mice

Mice knockout	Phenotype	Substrates	Ref.
global *Derl1* −/−	Die in *utero* at E7-E8.	not identified	[67]
global *Derl3*−/−	Mice were born and grew normally. Also exhibited decreased levels of Derlin-1 and Derlin-2 in the pancreas.	not identified	[67]
global *Derl2*−/−	Pups are born at normal Mendelian ratios, but majority dies within 24 hours after birth due to inability to feed. Few surviving mice developed skeletal dysplasia and exhibited dilated ER due to defects in collagen matrix protein secretion by chondrocytes. Also, all tissues were unaffected, but had upregulated levels of ER chaperones and exhibited chronic UPR.	*Derl2*−/− chondrocytes show ER retention of collagen matrix proteins.	[66]
B cells *Derl2*−/−	Der2 deficiency does not affect B cell development and antibody secretion.	not identified	[66]
Hepatocytes *Derl2*−/−	Cells have ongoing UPR, but does not affect liver function.	not identified	[66]
Schwann cells *Derl2*−/−	Impairment of ERAD in nerves, but no effect on developmental myelination or remyelination after nerve injury. Aged mice develop demyelinating neuropathy in which UPR fails to activate.	OS9 chaperone is stabilized and ER retention of Charcot- Marie-Tooth-associated myelin protein zero (P0-S63del).	[68]
Podocytes *Derl2*−/−	Mice were normal in appearance and behavior and exhibited negligible difference in kidney histology. These mice are susceptible to ADR-induced glomerular injury and death. This is accompanied by compromised ERAD and ER stress.	not identified	[114]
global *iRhom1*−/−	Die by 6 weeks of age.	TACE is retained in the ER in several tissue types.	[115]
global *iRhom2*−/−	Appear normal, but have impaired immune response.	TACE is retained in the ER in macrophages.	[115]

Table 2.

Rhomboid member's association with diseases

Rhomboid member	Disease	Disease link	Ref.
Derlin-1	Colon Cancer	Derlin-1 silencing led to growth inhibition and promoted apoptosis in colon cancer cells. Derlin-1 overexpression correlates with tumor differentiation, invasion, and metastasis.	[80]
Derlin-1	Breast Cancer	High expression of Derlin-1 correlated with tumor grade and metastasis. Derlin-1 silencing sensitized breast cancer cells to ER stress-induced apoptosis.	[79]
Derlin-3	Breast Cancer	Inhibiting Derlin-3 expression decreased BC proliferation and high DERL3 expression in patients experienced poorer prognosis.	[77]
Ubac2	Becget disease	Two risk alleles, rs9517723 and rs7999348, significantly correlates with enhanced UBAC2 expression, which contributes to CNS lesions in patients. However, one risk allele, rs3825427, is correlated with downregulated UBAC2.	[96]
Rhbdl4	Colorectal Cancer	High expression of Rhbdl4 is associated with metastatic colorectal cancer. Its role in colorectal cancer has been proposed to be through the Wnt and EGFR signaling pathways.	[108–110]
Rhbdl4	Glioblastoma	Knockdown of Rhbdl4 resulted in an increase in apoptosis in a glioblastoma derived cell line.	[111]
iRhom1	Breast Cancer	Elevated expression of iRhom1 was found to be correlated with breast cancers with a faster progression. Possible mechanism is by promoting stability of transcription factor Hif1α, thus promoting cellular adaptation to hypoxia.	[100]