Intramembrane proteolysis controls diverse signalling pathways throughout evolution

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Regulated intramembrane proteolysis (RIP) has recently emerged as a conserved mechanism for controlling many signalling pathways in both prokaryotes and eukaryotes. Although early examples were confined to the activation of membrane-tethered transcription factors in the cell receiving the signal, recent analysis indicates that RIP also regulates the emission of factors involved in intercellular communication.

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Abbreviations

APP	Alzheimer precursor protein
ATF6	activating transcription factor 6
EGF	epidermal growth factor
EGFR	EGF receptor
ER	endoplasmic reticulum
MHC	major histocompatibility complex
RIP	regulated intramembrane proteolysis
S2P	site 2 protease
SP	signal peptide
SPP	signal-peptide peptidase
SREBP	sterol response element binding protein
TMD	transmembrane domain
UPR	unfolded protein response
XBP-1	X-box binding protein 1

Introduction

There is growing evidence that intramembrane proteolysis is a widely conserved mechanism for controlling diverse biological processes [1••,2]. The substrates that undergo such proteolysis are transmembrane proteins that are inactive in their membrane-tethered form, usually by virtue of being physically sequestered from their sites of action. Intramembrane proteolysis results in the cleavage of these proteins within their transmembrane domains (TMDs), which thereby releases their cytoplasmic or luminal/extracellular domains and allows them to translocate to a new location where they can elicit the biological response. Intramembrane proteolysis is therefore a way of controlling physiological processes by the regulated release of sequestered protein domains that become free to act at a distance. Because intramembrane proteolysis is the trigger that activates the signalling process, it is precisely regulated; this has led to the term 'regulated intramembrane proteolysis' (RIP) [1••].

Four protease families are currently known to catalyse intramembrane proteolysis: the presenilins [3,4] and the site 2 protease (S2P) family [5,6] were described first, whereas the rhomboids [7••] and the signal-peptide peptidase (SPP) [8••] were isolated only very recently (Figure 1). Despite their diversity, these unusual proteases are characterized by three common features. First, each protease is a polytopic membrane protein with catalytic residues located in different TMDs; these residues then associate to form the protease active sites in the membrane bilayer [3,5,7••,8••]. Second, although these proteases have no evolutionary sequence similarity to other known proteases, they have short sequence motifs surrounding their catalytic residues that are typical of traditional proteases of the same mechanistic class [5,7••,8••,9•]. This probably reflects the convergent evolution of catalytic centres towards an optimal function. Last, the proteases occur in large protein families that are typically conserved from archaea to humans [5,6,8••,10,11•].

The remarkable diversity and conservation of RIP suggests that it is an ancient control mechanism that evolved early and has been adapted in different organisms to regulate many different biological processes [1••] (Table 1). Current evidence suggests that the predominant function of this form of proteolysis is the regulation of cell signalling. In fact, one of the most striking insights is the apparent conservation of these signalling mechanisms (including intercellular signalling) between metazoans and prokaryotes (Figure 2). In general, the signals that are involved in RIP-mediated signalling are either intracellular cues or chemical signals that are produced by other cells. In this respect, two types of cell can be classified: signal-receiving cells that respond to signals, and signal-emitting cells that actively produce signals for communication with other cells. Although RIP was originally defined by the activation of membrane-tethered transcription factors in response to various signals [1••,2], recent analysis has established that RIP is involved more broadly in regulating the activation of factors involved in both signal reception and signal emission.

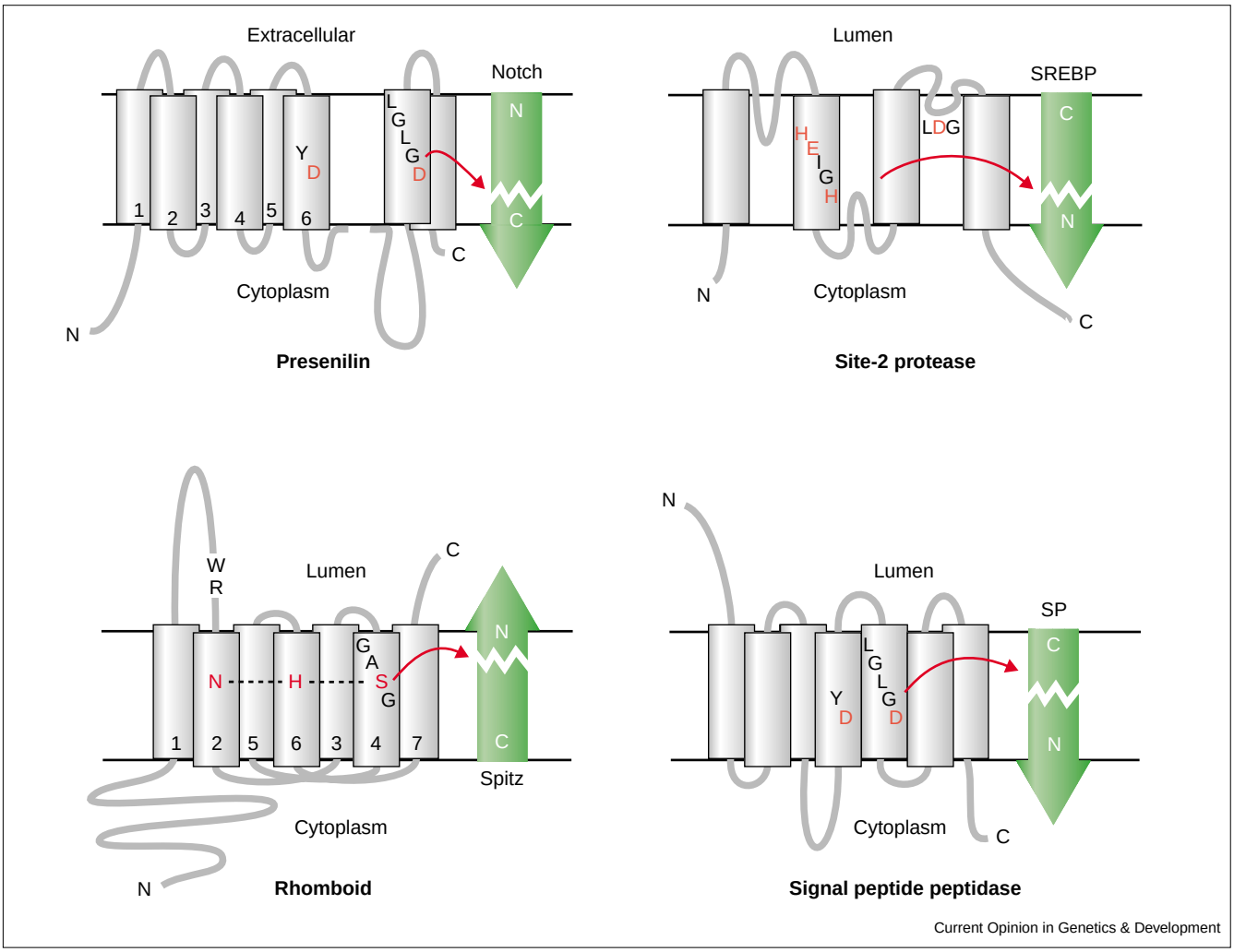
Signal reception by RIP

Presenilin family

Presenilins are a family of intramembrane aspartyl proteases that are the catalytic component of the γ-secretase complex [3,4,12••,13••] (Figure 1). RIP mediated by presenilin regulates cellular responses to several unrelated signals, in most cases by releasing membrane-tethered transcription factor domains [14]. This cleavage can be either the first event in regulating the transduction of the signal from the membrane to the interior of the cell or a much later response to secondary messengers as part of a more complex transduction system.

Cell-surface receptors can be cleaved directly by presenilin after ligand binding and ectodomain cleavage (Table 1).

Figure 1



Representation of the four known families of intramembrane proteases. Catalytic residues are in red, and conserved motifs that are typical of proteases of each mechanistic class are in black. Note that presenilins and Rhomboids cleave only type I proteins (with an extracellular/luminal N terminus), whereas site 2 proteases and signal-peptide peptidases cleave type II proteins. The topology of each protease has been determined experimentally. The approximate sites of cleavage are shown, and the green arrow indicates the direction of domain release: only Rhomboids are involved predominantly in extracellular release of factors. Presenilins and signal-peptide peptidases are aspartyl proteases that use two aspartates to cleave substrates, the site 2 proteases are metalloproteases that coordinate a zinc ion using two conserved histidines and an aspartate, and Rhomboids are serine proteases that use a catalytic triad to cleave substrates (hydrogen bonds of the triad are indicated as dashed lines).

The first described and best characterised of these is the Notch receptor, which controls many aspects of animal growth and development. Presenilin cleavage of Notch liberates its cytoplasmic transcriptional activator domain, thereby allowing Notch to enter the nucleus and activate transcription of its target genes [15–17]. Although Notch proteolysis by presenilin represented a new model for transducing signals from receptor to nucleus, recent evidence suggests that it may be a more general mechanism. For example, the ErbB4 receptor tyrosine kinase has been recently shown to be cleaved by presenilin in response to binding of the growth factor heregulin, an epidermal growth factor (EGF) ligand [18•,19•]. The cleaved ErbB4 intracellular domain enters the nucleus, where it shows some transcriptional activity, and is required for the biological outcome of ErbB4 signalling in a tissue culture model. It is currently not clear how ErbB4 signal transduction by RIP relates to much better characterised transduction by cytoplasmic pathways such as the Ras/mitogen-activated protein kinase (MAPK) and phoshphatidylinositol-3-OH kinase pathways [20]; for example, what is the physiological contribution of each transduction mechanism to the final outcome of ErbB4 signalling?

Presenilins can also cleave other targets such as Alzheimer precursor protein (APP) and the cell-adhesion molecule E-cadherin (Table 1). Indeed, it was the study of individuals with familial Alzheimer disease that led to the identification

Table 1

The diverse roles of RIP in signalling.

Protease	Pathway	Primary signal	RIP in signal-receiving cell	RIP in signal-sending cell	Refs
Presenilins	Notch signalling	Notch ligands (Delta and Serrate)	RIP of Notch receptor (TF release)		[15,16]
	EGF signalling	EGF ligands (e.g. Heregulin)	RIP of ErbB4 receptor (TF release)		[18•,19•]
	2nd messenger signalling (e.g. Ca^{2+}, PKC)	Various	RIP of APP (TF release, other roles?)		[24•–26•]
	2nd messenger signalling (e.g. Ca^{2+}, apoptosis)	Various	RIP of E-cadherin (junction disassembly, β-catenin TF release?)		[27]
SP family	Cholesterol/palmitate biosynthesis	Low cholesterol, low palmitate	RIP of SREBPs (TF release)		[28–30,31•,32•]
	Unfolded protein response	Unfolded proteins	RIP of ATF6 (TF release)		[33•]
(SpolVFB)	Sporulation (*B. subtilis*)	SpolVB	RIP of pro-σ^K (TF release)		[6]
(eep)	Sex pheromone (*E. faecalis*)	cAD1 pheromone (positive signal)		RIP of lipoprotein SP (cAD1 release)	[52]
		iAD1 inhibitor (negative signal)		RIP of SP-like precursors (iAD1)	[53]
Rhomboids	EGFR signalling (*Drosophila*)	EGF ligands (Spitz, Keren, Gurken)		RIP of Spitz, Keren, Gurken (EGF ligand release)	[7••,41–43]
(AarA)	Quorum sensing (*Providencia*)	Unknown peptide factor		Release of unknown peptide signal	[46,47,49•]
SPP family	HLA-E signalling	MHC SP fragment		RIP of MHC SPs	[59]
	2nd messenger signalling	Various	RIP of SPs (cytoplasmic release of peptides that alter signal reception?)		[60]

APP, Alzheimer precursor protein; EGF, epidermal growth factor; MHC, major histocompatibility complex; PKC, protein kinase C; RIP, regulated intramembrane proteolysis; S2P, site 2 protease; SP, signal peptide; SPP, signal-peptide peptidase; SREBP, sterol response element binding protein; TF, transcription factor.

of the presenilins [14,21]; processing of APP by presenilins generates the Aβ42 peptide, which forms senile plaques in individuals affected with Alzheimer disease. Processing of APP can be regulated by protein kinase C and other secondary messengers [22,23], and recent work suggests that the cytoplasmic domain released by RIP enters the nucleus, where it can modulate transcription [24•–26•]. In this respect, the release of APP cytoplasmic domain may also be a form of signal transduction in response to secondary messengers. In addition, cell adhesion may be regulated by RIP of E-cadherin by presenilin in response to secondary messengers such as calcium influx and apoptosis cascades [27], although the exact physiological significance of this observation remains to be determined. The site of E-cadherin cleavage was unexpectedly mapped to the membrane/intracellular interface, which suggests that cadherin cleavage may be due to an indirect effect, rather than direct RIP of E-cadherin by presenilin.

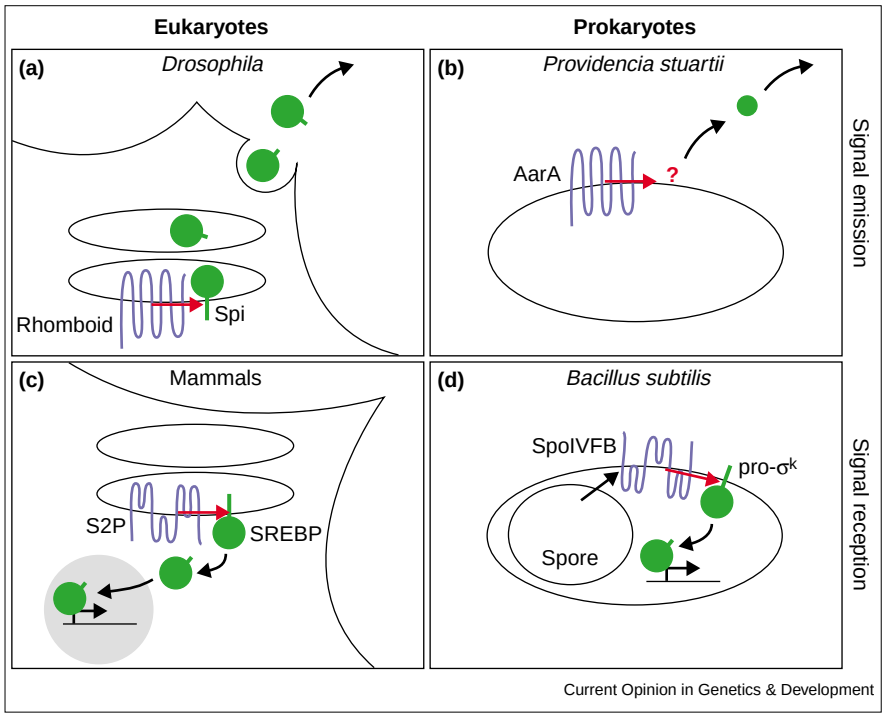
Homologues of presenilin have been discovered recently in simple organisms such as certain archaeal species [11•]. Extending analyses to these organisms should provide valuable insight into the biological roles and evolution of this form of RIP.

S2P family

In metazoans, RIP mediated by the S2P metalloprotease family occurs in response to cues that monitor the integrity of the endoplasmic reticulum (ER), sensing both lipid and protein composition (Table 1). S2P itself activates cholesterol biosynthesis in mammals. The signal is reduced levels of cholesterol

Figure 2

Control of cell signalling by regulated intramembrane proteolysis in eukaryotes and prokaryotes. **(a,b)** Rhomboid mediates the release of signals for communication with other cells. The *Drosophila* EGFR ligand Spitz is cleaved in the Golgi apparatus and subsequently secreted. Note that the signal in *Providencia* seems to be a small peptide, but its identity is not known (represented by a question mark). **(c,d)** Site 2 protease mediates signal reception by releasing transcription factors that activate genes involved in cholesterol biosynthesis in mammals or in late sporulation in the mother cell of *B. subtilis*.



in ER membranes, which triggers the translocation of the sterol response element binding proteins (SREBPs) from the ER to the Golgi apparatus by the SCAP (SREBP cleavage activating protein) transport factor [28,29]. This translocation ultimately leads to RIP of the membrane-tethered transcription factor by S2P in the Golgi apparatus [30], which thereby allows them to activate the genes for cholesterol biosynthesis (Figure 2). Notably, the control of cholesterol biosynthesis may be a relatively recent adaptation of this signalling mechanism: *Drosophila* has homologues of SREBP, S2P and other regulatory proteins, but insects cannot synthesize sterols and must obtain them from their diet. The *Drosophila* S2P pathway is triggered by phosopholipids containing low amounts of palmitate and it stimulates palmitate biosynthesis, which suggests that membrane integrity rather than cholesterol *per se* may have been the common evolutionary signal for this form of RIP [31•,32•].

The unfolded protein response (UPR) is also regulated by S2P-mediated RIP in metazoans [33]. Unfolded proteins in the ER stimulate translocation of the membrane-tethered activating transcription factor 6 (ATF6) from the ER to the Golgi apparatus [34], where it is cleaved by S2P. The liberated transcriptional activation domain enters the nucleus and, together with XBP1 (X-box binding protein 1) [19•,35], promotes the activation of genes involved in the UPR, including ER chaperones such as BiP and factors responsible for slowing protein synthesis [36]. It is not clear whether the role of S2P in sensing intracellular cues in metazoans is a true specialization of this form of RIP, or whether our knowledge simply reflects the predominant study of cell-culture models in which possible roles in intercellular communication would not have been detected. The genetic analysis possible in *Drosophila* should reveal the full spectrum of functions of S2P.

A role for S2P proteases in intercellular communication has also been demonstrated in prokaryotes. Sporulation in *Bacillus subtilis* is regulated by RIP of the membrane-tethered pro-σ^k factor in the mother cell, which then activates the genes for late sporulation (Figure 2). The responsible protease, SpoIVFB, encodes a homologue of S2P [6]. The signal secreted by the forespore is SpoIVB, which activates the SpoIVFB metalloprotease in the mother cell, resulting in RIP of pro-σ^k as a signal-reception mechanism [37]. Homologues of S2P have been detected in many other bacteria, but their function has begun to be addressed only recently; for example, knockout of the *Escherichia coli* S2P gene *yaeL* resulted in lethality with an abnormal cell elongation phenotype [38], although the mechanistic basis for this is not yet clear.

Signal activation by RIP

Until recently, the role of RIP in releasing membrane-tethered transcription factors was thought to be its sole function. Now intramembrane proteolysis has been shown to be involved in generating signals as well as receiving them, thereby considerably broadening the range of biological functions in which RIP has an important role (Table 1 and Figure 2).

Rhomboid family

Rhomboid is a novel protein with seven TMDs [39] that functions in the signal-sending cell to initiate epidermal growth factor receptor (EGFR) signalling in *Drosophila* [40].

Although the EGFR and its main activating ligand Spitz are expressed broadly during development, Rhomboid activity is limiting and its expression prefigures and activates signalling. Thus, in *Drosophila* Rhomboid is the trigger that spatially and temporally restricts EGFR signalling in its many developmental contexts.

Recent work has deciphered the mechanism of Spitz activation and identified it as the first known example of intramembrane proteolysis in growth factor release (Figure 2). Spitz is inert in its transmembrane form, being confined to the ER, and its activation is regulated by subcellular trafficking to the Golgi apparatus by the chaperone Star [41,42]. There, the EGF domain of Spitz is released by Rhomboid-mediated RIP and becomes secreted [7••]. Rhomboid is the first intramembrane serine protease to be described and uses a catalytic triad to cleave Spitz [7••] (Figure 1). All three membrane-tethered EGFR ligands in *Drosophila* are activated by the same RIP mechanism [43,44], and recent evidence suggests that at least four of the seven *Drosophila* Rhomboids are dedicated to EGFR activation [44]. But the whole spectrum of their functions within an organism remains to be determined.

Rhomboid belongs to a large gene family with currently ~100 members that are conserved throughout archaea, bacteria, yeast, plants and animals including humans [10,45]. Despite this wide conservation in almost all branches of life, only one Rhomboid has been studied outside *Drosophila*. The pathogenic Gram-negative bacterium *Providencia stuartii* monitors its population size by secreting a signalling factor that accumulates at higher cell densities and activates virulence genes [46,47]. This intercellular communication is termed 'quorum sensing' and is now known to be used by most if not all bacteria for different intercellular signalling events [48]. Remarkably, the *Providencia* Rhomboid, called AarA, was identified as being necessary for signal generation in quorum sensing [47]. Although the precise mechanism of this signalling remains to be determined, the signal seems to be a peptide [46], and the parallels with *Drosophila* Rhomboid function are striking [49•] (Figure 2).

In support of the conclusion that *Drosophila* and bacterial Rhomboids have related functions, AarA and many other bacterial Rhomboids can cleave all three *Drosophila* transmembrane EGFR ligands [50]. In addition, the activities of the *Drosophila* and *Providencia* Rhomboids are also similar in a biological context; AarA has been shown to cause EGFR hyperactivation phenotypes when ectopically expressed in flies, and the *Drosophila* Rhomboid could partially rescue the signalling defect in aarA-mutant *Providencia* (M Gallio, unpublished data). RIP mediated by Rhomboids may therefore be one of the first signal-activation strategies to have evolved for intercellular communication. Several rhomboids from vertebrates are also active intramembrane proteases [7••], and some have dynamic expression patterns [51] that are consistent with a role in developmental signalling. But preliminary evidence from yeast suggests that not all Rhomboids are involved in intercellular signalling (A McQuibban, M Freeman, unpublished data); like the other intramembrane protease families, their overall functions may turn out to be more broad.

S2P family

The S2P homologue of *Enterococcus faecalis* has also implicated RIP in bacterial intercellular communication [52]. The inert signal in this process is a cleaved signal peptide (SP) sequence from an unrelated protein [53,54]. Cleavage of this SP remnant releases the octapeptide pheromone cAD1 that governs sexual behaviour in neighbouring cells [55], including transfer of virulence factors and antibiotic resistance. Once the transfer has taken place, S2P cleavage then produces the inhibitor iAD1 by RIP of a different SP that is encoded by the transferred DNA [53].

This overall quorum-sensing mechanism seems to be conserved in related bacteria [54,56], although it is not yet clear whether it is widespread.

SPP family

In metazoans the fate of SPs after their cleavage from proteins translated in the ER has been poorly understood, but biochemical evidence suggests that they are removed by intramembrane proteolysis [57]. Very recently, a specific human SP peptidase (SPP) has been isolated biochemically and found to be a novel intramembrane protease with presenilin-like aspartyl protease signature motifs that surround its conserved active-site residues [8••]. Unlike other RIP protease families, SPP seems to be conserved predominantly in higher eukaryotes and may thus have a more specialized function in SP cleavage.

Although SP cleavage is thought to be primarily a scavenging event that removes these remnants, several of the released peptides seem to have a second function in regulating signalling events [58], for example, in the immune system [59]. The SPs of several class I major histocompatibility complex (MHC) proteins contain a conserved motif that, when released from the SPs by SPP, is displayed on the cell surface in complex with the HLA-E immune receptor. In addition to the MHC molecules themselves, these liberated MHC SPs act as signals that are recognized by natural killer cells, thus protecting the MHC-expressing cells from attack. But it is not clear whether this cleavage is regulated. SPP-cleaved SP fragments can also interact with cytosolic signalling proteins and could thus be involved in signal reception [60], although the physiological significance of this is not understood as yet.

Conclusions and perspectives

We have focused on the growing number of intramembrane proteases and their diverse roles in signalling both within and between cells. Although recent advances have expanded the roles and characteristics of RIP, many issues remain unresolved.

Although intramembrane proteolysis is used to control a vast range of biological contexts, the RIP proteases seem to show a bias towards being involved mainly in either signal reception

or signal activation. The proteases involved in signal activation tend to have active sites closer to the extracellular side of the membrane, perhaps to facilitate signal release, whereas the converse is true for those involved in signal reception through transcription factor release into the cytosol (Figure. 1). It remains to be seen whether this bias represents a significant evolutionary or functional division.

Regulated intramembrane proteolysis — the term coined for these events — emphasizes the regulated nature of the cleavage. For the early examples of presenilin and S2P RIP, a prior cleavage that removes the target protein ectodomain regulates the subsequent RIP cleavage. More recent examples suggest that in some cases the enzyme activity is not itself subject to such post-translational regulation. For example, *Drosophila* Rhomboid activity seems to be regulated solely by its transcription: if Rhomboid is present then cleavage occurs (S Urban, unpublished data). Further analysis is required to determine how SPP activity and the activity of some prokaryotic intramembrane proteases is regulated.

There are important issues concerning how the hydrolysis reaction occurs in the naturally hydrophobic environment of the lipid bilayer. Two classes of model have been proposed: either the multiple TMDs that are essential elements of intramembrane proteases may provide a hydrophilic pocket to allow access to the water needed for hydrolysis, or there may be a transient contortion of the substrate TMD, which stretches it out of the bilayer. These issues are unlikely to be resolved unambiguously until the intramembrane proteases yield to enzymatic and structural analysis, which is a major future challenge for the field.

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