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Review

Function of site-2 proteases in bacteria and bacterial pathogens ☆

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ABSTRACT

Site-2 proteases (S2Ps) are a class of intramembrane metalloproteases named after the founding member of this protein family, human S2P, which control cholesterol and fatty acid biosynthesis by cleaving Sterol Regulatory Element Binding Proteins which control cholesterol and fatty acid biosynthesis. S2Ps are widely distributed in bacteria and participate in diverse pathways that control such diverse functions as membrane integrity, sporulation, lipid biosynthesis, pheromone production, virulence, and others. The most common signaling mechanism mediated by S2Ps is the coupled degradation of transmembrane anti-Sigma factors to activate ECF Sigma factor regulons. However, additional signaling mechanisms continue to emerge as more prokaryotic S2Ps are characterized, including direct proteolysis of membrane embedded transcription factors and proteolysis of non-transcriptional membrane proteins or membrane protein remnants. In this review we seek to comprehensively review the functions of S2Ps in bacteria and bacterial pathogens and attempt to organize these proteases into conceptual groups that will spur further study. This article is part of a Special Issue entitled: Intramembrane Proteases.
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1. Introduction

Site-2 proteases (S2Ps) are widely distributed in bacteria and participate in diverse pathways, all of which share the requirement for proteolysis of a transmembrane protein. For the purpose of this review, we define S2Ps as multipass transmembrane proteins with a conserved zinc metalloprotease active site (HExxH) within a transmembrane domain and an xDG motif within another transmembrane domain [1,2]. Many bacterial S2Ps also have a centrally located PDZ domain [1]. Due to their prominence as model organisms, the S2Ps of *Escherichia coli* (RseP) and *Bacillus subtilis* (YluC and SpoIVFB) have been intensely studied and are the best understood in terms of upstream activating

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signals, signal transduction mechanism, and downstream regulons (Table 1). Expanding investigation of S2Ps in bacterial pathogens has revealed roles for S2Ps in sensing host signals and regulating virulence gene expression during infection. In most cases, the signaling cascades in which S2Ps participate follow the same general paradigm. A site-1 protease (S1P) first cleaves the (usually) extracytoplasmic segment of the transmembrane substrate in response to specific inducing signal (e.g. unfolded outer membrane proteins in the case of the S1P (DegS) in the *E. coli* SigE pathway). This site-1 cleavage is rapidly followed by S2P cleavage within the transmembrane segment of the substrate, thereby liberating the cytosolic fragment of the substrate. In many cases, the fragment released into the cytosol by the S2P cleavage is a transcriptional regulator. Although the tight coupling between S1P and S2P cleavage events is a hallmark of many of these signaling systems, the mechanisms that link S1P and S2P cleavage are still poorly understood.

Despite wide distribution of the S1P/S2P signaling paradigm, variations on this theme continue to emerge as more S2P signaling systems are studied. First, there is great diversity in both the signals that induce the S1P cleavage event and the physiologic consequences of pathway activation. In many cases, the S1P/S2P cleaved transmembrane protein is a transcriptional regulator, but the proteolytic destruction of the regulator has diverse effects depending on the system, in some cases activating gene expression and in some cases repressing. Finally, although the S1P/S2P paradigm of transmembrane signal transduction is widely distributed, exceptions to the rule continue to emerge, including examples in which S2P mediated cleavage occurs apparently independently of a S1P, examples in which S2P cleavage releases a extracellular signaling molecule rather than a cytoplasmic fragment, and functions for S2Ps in general cleavage of signal sequence remnants. In this review, we will systematically review S2P systems in bacteria and bacterial pathogens with the goal of highlighting canonical prokaryotic S2P systems and the deviations from the canon. This review benefits heavily from other recent outstanding reviews of this field, to which we refer the reader for additional details and perspectives [3–6].

2. Bacteria

2.1. E. coli RseP

The *E. coli* sigma factor E (SigE) periplasmic stress response pathway is one the best studied S2P containing signaling systems. SigE is an alternative sigma factor of the extracytoplasmic function (ECF) sigma factor subclass, termed as such because these sigma factors typically respond to and regulate extracytoplasmic processes [7–9]. The SigE pathway of *E. coli* follows the common paradigm of a three component S1P/anti-sigma factor/S2P system (Fig. 1A). The SigE ECF sigma factor is held inactive by the RseA transmembrane anti-sigma factor. The upstream activating signals for the pathway are the C-terminal hydrophobic amino acids of β-barrel outer membrane proteins (OMPs), which are ordinarily sequestered, but become exposed under conditions that unfold OMPs such as heat shock. The C-terminal peptides of OMPs bind to the DegS PDZ domain, thereby activating DegS to cleave the periplasmic domain of RseA [10–14]. RseB also controls DegS proteolysis of RseA by binding directly to the periplasmic domain of RseA [10]. DegS cleaved RseA is a substrate for RseP [15–17], although the mechanism of coupling of these two proteolytic events remains controversial [18,19]. Following RseP cleavage, the RseA/SigE complex is released to the cytoplasm where further degradation of RseA [20] releases SigE to associate with RNA polymerase to activate target promoters. The SigE regulon, which is essential, includes genes involved in cell envelope remodeling, chaperones, and the heat shock response [21,22]. The RseP pathway is one of the best understood signal transduction pathways involving a S2P and forms the paradigmatic example of a transmembrane signaling system in which an ECF sigma factor is activated by coupled proteolytic destruction of a transmembrane anti-sigma factor (Illustrated in Fig. 1A).

2.2. B. subtilis

2.2.1. YluC/RasP

The *B. subtilis* SigW pathway controls the availability of ECF sigma factor SigW. SigW is held inactive by the transmembrane anti-Sigma factor RsiW. The SigW pathway of *B. subtilis* conforms to the common theme of sequential proteolytic destruction of a transmembrane anti-sigma factor. The transmembrane anti-sigma factor RsiW (anti-SigW) is first cleaved by the S1P PrsW (also called YpdC), which is a novel site-1 protease not homologous to DegS [23,24]. Recent evidence also indicates that a second proteolytic event further shortens the PrsW cleaved RsiW [25]. The C-terminally shortened RsiW is then cleaved by the S2P YluC/RasP [26], which releases the cytoplasmic fragment of RsiW bound to SigW. The mechanism(s) by which the inducers of the SigW pathway are coupled to the activation of site-1 proteolysis of RsiW are not well understood. PrsW has also been implicated in the degradation of anti-sigma factors and virulence in *C. difficile*, although the S2P involved in this pathway has not been defined [27].

The SigW regulon is induced by a variety of signals, many of which perturb cell wall integrity. For example, induction of a SigW dependent promoter was observed with a variety of cell wall inhibiting antimicrobials, including Beta lactams and Vancomycin [28]. Similarly, antimicrobial peptides activate the SigW regulon [24], as does alkaline shock (pH 8.9) [29]. The SigW regulon consists of approximately 30 operons [30]. Consistent with its role in responding to perturbations in membrane integrity, one SigW-responsive promoter lies within the *fabHa-fabF* locus and is induced by alkaline shock, cell wall active antibiotics, and TritonX-100 [31]. Upregulation of this promoter increases membrane rigidity through upregulation of FabF and downregulation of FabHa, resulting in longer chain fatty acids, thereby increasing membrane rigidity [31].

A second substrate for YluC/RasP has been reported. FtsL, a transmembrane protein involved in cell division, is rapidly degraded in wild type cells but stabilized by inactivation of *yluC* [32]. The YluC-FtsL cleavage reaction was recapitulated in *E. coli,* supporting a direct proteolytic relationship between YluC and FtsL [32]. In addition to showing that the S2P YluC has two substrates, FtsL cleavage by YluC does not apparently require a site-1 cleavage event. The function of YluC in this

Table 1
Signaling systems in bacteria mediated by S2Ps. Listed is the bacterial species, the S1P (if known or required), the substrate of the S2P, and the S2P itself. If the S2P signaling system controls a transcriptional regulator, it is listed along with the physiologic function of the pathway and relevant references.

Bacterium	S1P	Substrate	S2P	Transcriptional regulator	Activating Signal/Pathway	Refs.
E. coli	DegS	RseA	RseP	SigE	C termini of unfolded OMPs/Cell envelope stress response	[10,12–14,16,17,21]
B. subtilis	PrsW + TSP	RsiW	YluC/RasP	SigW	Alkaline Shock, antimicrobial peptides, cell wall active antimicrobials/ Membrane rigidity	[23–26,28,30,31]
B. subtilis	SpoIVB (conceptually, see text)	pro-SigK	SpoIVFB	SigK	Sigma G activation in forespore / Latter stages of sporulation developmental program in mother cell	[2,35,41]
Caulobacter crescentus	PerP	$PodJ_{L,}$ -$PodJ_s$	MmpA	none	Cytokinesis/Polar cell division	[49,50]

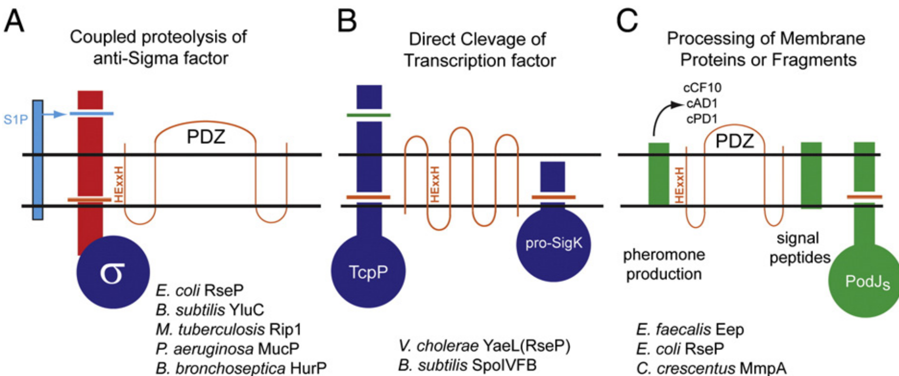

Fig. 1. Conceptual groupings of S2P mediated signaling pathways. A. Coupled Proteolysis of transmembrane anti-Sigma factors. In this widely distributed mechanism of ECF Sigma activation, an activating signal triggers Site one proteolysis (S1P, light blue) of the periplasmic domain of the anti-Sigma, which is rapidly followed by S2P mediated cleavage in the transmembrane segment (orange cut). The mechanism of this tight coupling is still poorly understood. S2Ps of this type contain PDZ domains and include the S2Ps listed below the figure. B. Direct Cleavage of a transcriptional regulator. In this mechanism, the proteolytic target is a membrane bound transcriptional regulator, either TcpP or pro-SigmaK. In the case of TcpP, which is cleaved by YaeL(RseP), the cleavage event shuts off gene expression. In the case of pro-SigK, proteolysis (by SpoIVFB) liberates the active transcription factor. Note that the transmembrane topology pictured is that of SpoIVFB, not RseP (which is represented in Panel A). C. Cleavage of membrane proteins or protein remnants. In this grouping, the S2P processes a membrane protein that is not a transcription factor. Examples include the production of peptide pheromones in *E. faecalis* from lipoprotein signal sequences by Eep, the processing of signal peptides by RseP, and the destruction of PodJs by MmpA in *C. crescentus*. The constitutive cleavage of FtsL by YluC also may fall into this category. See text and Tables for details.

pathway is constitutive degradation of FtsL, rather than proteolysis upon activation by an upstream protease. This latter feature may suggest that the mechanisms by which most S2Ps are held inactive until site-1 cleavage occurs may not be operative for the YluC/FtsL pair.

There is yet another predicted transmembrane anti-Sigma factor in *B. subtilis*, RsiV, which is paired with SigV. This pathway is induced by lysozyme, but the role of RsiV proteolysis in this cascade has not yet been examined [33,34].

3. SpoIVFB-pro-SigK pathway

SpoIVFB is a founding member of the bacterial S2P metalloprotease family that plays a crucial role in the latter stages of the sporulation program [2,35]. SpoIVFB cleaves pro-SigK, a membrane associated transcription factor, to a mature, active isoform, SigK, that governs gene expression in the mother cell after the forespore engulfment stage of *Bacillus* sporulation [2,36]. Whereas SigK ultimately controls the transcriptional program in the mother cell, the signal for pro-SigK activation originates in the forespore in the form of SigG-dependent expression [37] and subsequent secretion of serine protease, SpoIVB [38]. Like RseP and many other S2Ps, SpoIVFB is held inactive until the appropriate activation signal arrives, in this case SpoIVB from the forespore. Two proteins, BofA and SpoIVFA, form a stable complex with SpoIVFB in the outer forespore membrane (OFM) and inhibit cleavage of pro-SigK [36,37,39,40]. SpoIVB is secreted from the forespore to the OFM where it degrades SpoIVFA, thereby relieving its inhibitory effect on SpoIVFB [41]. SpoIVB also activates a second forespore-derived protease, CtpB, which can also cleave SpoIVFA, providing a redundant mechanism to activate SpoIVFB processing of pro-SigK [41–43]. Once BofA and SpoIVFA inhibition have been relieved, SpoIVFB cleaves between residues 20 and 21 on pro-SigK, which associates with the OFM through an N-terminal transmembrane domain (residues 1–27), releasing soluble SigK [44]. This degradation is dependent upon the presence of ATP which may interact with SpoIVFB to alter its interaction with pro-SigK in a manner that facilitates pro-SigK cleavage [45]. SigK then directs gene expression in the mother cell in the latter stages of the developing sporangial cell, including genes for spore cortex, coat synthesis, and ultimately mother cell lysis and mature spore release [39]. Thus, SpoIVFB is the linchpin in the final sigma factor checkpoint between forespore and mother cell prior to the last stage of sporulation.

The *B. subtilis* SpoIVFB pathway differs from the aforementioned paradigmatic S1P/ Substrate/S2P systems in several important ways. In contrast to RseP, the SigK-SpoIVFB cascade provides an example a S2P cascade in which the intramembrane cleavage event occurs in the transcriptional regulator itself, rather than an anti-sigma factor (Illustrated in Fig. 1B). Furthermore, there is no S1P that directly cleaves pro-SigK before SpoIVFB cleavage. However, a proteolytic cascade conceptually analogous to site-1 proteolysis does occur in the pro-SigK cascade. As has been suggested [41], one could think of the SpoIVB protease as a S1P that, instead of cleaving the S2P substrate to activate the cascade, cleaves an inhibitor in complex with the S2P. Finally, SpoIVFB also differs topologically from other S2Ps in its lack of a PDZ domain and the presence of six transmembrane domains (in contrast to the more common topology of 4 TMs) [46].

3.1. *Caulobacter crescentus*

C. crescentus PodJ is a localization factor involved in polar morphogenesis which exists in two isoforms, $PodJ_L$ and $PodJ_s$ [47,48] . The long form, $PodJ_L$, localizes to the swarmer cell pole in the predivisional cell. As the cell divides, $PodJ_L$ is replaced by $PodJ_s$, a truncated form of $PodJ_L$ that lacks the C terminus. The conversion of $PodJ_L$ to $PodJ_s$ occurs through proteolysis of the C terminal domain of PodJ by the periplasmic protease PerP at the time of cell division, and is regulated by cytokinesis signals via the DivJ–PleC–DivK system [49]. $PodJ_s$ persists at the flagellated pole of the post divisional cell, where it is required for chemotaxis [48].

MmpA is a S2P in Caulobacter that contains the canonical zinc metalloprotease active site and a centrally located PDZ domain. In a Δ*mmpA* strain of Caulobacter, stability of $PodJ_L$ is unaffected, but $PodJ_s$ is stabilized [50], consistent with MmpA degrading $PodJ_s$ in its transmembrane domain during the swarmer-to-stalked transition. In addition, MmpA can functionally complement a Δ*yaeL* strain of *E. coli* with respect to RseA degradation [50].

The *Caulobacter* PerP/PodJ/MmpA system in some ways follows the canonical S1P/TM substrate/S2P model, but differs in some important respects. In most of the S1P/S2P systems which cleave anti-sigma factors, the S1P/S2P cleavage events are tightly coupled and the S1P cleaved protein is a transient intermediate that only accumulates when the S2P is missing. In Caulobacter, $PodJ_s$, the product of the S1P (PerP) cleavage, is not only detectable, but has a distinct physiologic function in the cell [48]. These distinct functions for the two PodJ isoforms mandate that PerP and MmpA not be tightly coupled, as occurs in many other S1P/S2P systems. Another distinct feature of the Caulobacter system is the lack of apparent function of the cytoplasmic fragment of PodJ

that is released into the cytoplasm by MmpA cleavage of $PodJ_s$, implying that the function of the S2P in this system is to eliminate the membrane bound $PodJ_s$, rather than supply an active soluble fragment of the protein.

4. Bacterial pathogens

4.1. Mycobacterium tuberculosis Rip1

The *M. tuberculosis* (*Mtb*) genome encodes three putative S2Ps: Rv0359 (Rip2), Rv2625c (Rip3), and Rv2869c (Rip1), none are which are singularly essential for growth *in vitro* [51]. Initial examination of the Δ*rip1* strain of *M. tuberculosis* (but not Δ*rip2* or Δ*rip3*) demonstrated defects in cording, a macroscopic colonial morphology of *M. tuberculosis* associated with virulence and cell envelope lipid composition. Indeed, the Δ*rip1* mutant is defective in the localization of the three principal mycolic acids species to the outer *M. tuberculosis* cell envelope and displays complex perturbation of multiple lipid biosynthetic and catabolic genes at the transcriptional level [51,52]. In the mouse model of aerosol *M. tuberculosis* infection, deletion mutants in either *rip2* or *rip3* display similar growth kinetics to wild type *M. tuberculosis* (unpublished data) whereas *M. tuberculosis* Δ*rip1* is significantly attenuated in both the acute and chronic infection. Specifically, Δ*rip1* titers in mouse lung are 100 fold lower than wild type during acute infection and further decline 100 fold during chronic infection, indicating an important function for Rip1 in *Mtb* virulence [51].

Subsequent characterization of the Rip1 pathway has shown that, like many prokaryotic S2Ps, Rip1 cleaves the transmembrane anti-sigma factors for three ECF sigma factors: SigK, SigL, and SigM [52] (Table 2). The S1Ps that cleave any of the anti-sigmas of the Rip1 pathway are unknown. A candidate gene approach that examined several transmembrane proteases as candidate S1Ps in *M. tuberculosis* failed to identify the S1Ps of the Rip1 pathway [52].

Dissection of the relationships between Rip1 and these three Sigma factors regulons has indicated Rip1 dependent/SigKLM dependent pathways and Rip1 dependent/SigKLM independent pathways. Analysis of the transcriptomes of Δ*rip1*, Δ*sigK*, Δ*sigL, and* Δ*sigM* revealed that induction of the gene encoding the catalase-peroxidase KatG and its upstream iron dependent repressor FurA is dependent on Rip1, SigK and SigL [52]. By contrast, several other genes whose wild type expression pattern required Rip1 were not affected by loss of SigK, SigL, or SigM. The gene encoding the resuscitation-promoting factor C (*rpfC*), believed to play a role in dormancy, and the mycobacterial β-ketoacyl ACP synthase *kasA*, were both underexpressed in Δ*rip1* but unaffected by any sigma factor deletion [52]. These experiments strongly suggested that Rip1 controls pathways apart from the SigK, SigL, or SigM regulons, and by extension has additional substrates. This conclusion has been further substantiated by our recent finding that an *M. tuberculosis* Δ*sigK*Δ*sigL*Δ*sigM* triple mutant does not recapitulate the Δ*rip1* mutant phenotype in mice (M. Glickman, unpublished data).

One additional substrate of Rip1 has been identified in the literature, PBP3. PBP3 interacts with Wag31 (DivIVA), but when a mutant Wag31 protein is present that does not interact with PBP3, PBP3 is unstable and degraded by Rip1 under conditions of oxidative stress [53]. This may suggest that under certain circumstances in which the Wag31-PBP3 interaction is disrupted in wild type cells, Rip1 may degrade PBP3. This result also emphasizes the relative promiscuity of cleavage by Rip1, a feature shared with many S2Ps, which will cleave a variety of transmembrane segments without an apparent conserved cleavage site.

In summary, the Rip1 S2P controls an important virulence pathway in *M. tuberculosis* that regulates iron storage, lipid metabolism, oxidative stress defense (*katG*), dormancy (*rpfC*), and likely additional pathways that are yet to be defined. The major outstanding questions in the Rip1 pathway include the identity of the S1Ps, additional Rip1 substrates that contribute to the virulence phenotype of the Δ*rip1* strain, and the specific host signals that serve as the upstream activating signals of the Rip1 pathway *in vivo*.

4.2. Pseudomonas aeruginosa

P. aeruginosa is a gram negative opportunistic pathogen that causes a variety of clinical syndromes, including chronic lung infections in cystic fibrosis patients. *P. aeruginosa* can undergo mucoid conversion, a morphologic colony phenotype that is caused by overproduction of the polysaccharide alginate. Alginate production is controlled by an anti-sigma/sigma factor system in which the transmembrane anti-sigma (MucA) holds the sigma factor (AlgU) inactive. AlgU activates transcription of the genes encoding alginate biosynthetic enzymes [54–57]. MucA is subject to proteolytic destruction through sequential proteolysis by a S1P/S2P that closely resembles the *E. coli* RseA system. For a comprehensive review of this system, please see [4]. In *P. aeruginosa*, the S1P is AlgW [58] and the S2P is MucP. Accumulation of envelope proteins (MucE) activates AlgW (S1P) to cleave MucA in a manner similar to DegS cleaving RseA [58]. MucP then cleaves MucA within its transmembrane domain, releasing the MucA/AlgU cytoplasmic complex [58–60]. The upstream activating signals of the alginate pathway include unfolded membrane proteins (similar to the *E. coli* SigE pathway) [58] and cell-wall active antibiotics [61].

Although the AlgW/MucA/MucP cascade follows the standard model of other S1P/Anti-Sigma/S2P systems, there is evidence that MucA proteolysis by MucP can occur independently of AlgW under certain conditions. *Pseudomonas* strains isolated from cystic fibrosis patients often display a constitutive mucoid phenotype. Some of these strains have a nonsense mutation in MucA which truncates the protein. This truncated MucA (MucA22) does not require AlgW proteolysis, but may require MucP degradation [4]. Similarly, in the absence of MucD, MucA activation is AlgW independent but MucP dependent [59]. These findings are thus similar to the S1P-independent proteolysis of anti-sigma factors that occurs when C-terminally truncated forms are expressed in *M. tuberculosis* and *E. coli* [16,52]. In addition, some evidence suggests that MucP can directly degrade MucA in the absence of AlgW in wild type cells [4,59,62].

There is also evidence that MucP participates in other signaling systems apart from Alginate biosynthesis. One report [63] suggests that MucP in *Pseudomonas* cleaves FpvR, an anti-sigma factor for the sigma factors PvdS and FpvI. PvdS and FpvI regulate in iron uptake through siderophore biosynthesis [63]. These results indicate that the Pseudomonas MucP S2P has multiple substrates that presumably use independent S1Ps for activation.

4.3. Bordetella bronchiseptica

B. bronchiseptica is a respiratory pathogen that infects agricultural animals and occasionally humans and is related to the major human pathogen *B. pertussis*, the cause of Whooping Cough. *B. bronchiseptica hurP* encodes an S2P necessary for heme utilization [64]. *hurP* can complement the *Vibrio cholerae yaeL* null mutant, which cannot degrade TcpP [64]. Though *hurP* is not essential in *Bordetella,* it is required for heme utilization as the sole source of nutrient iron through induction of the outer membrane Heme receptor BhuR. The substrate of HurP that controls this pathway has not been directly identified, but is likely HurR, a predicted transmembrane protein that may act as the anti-Sigma factor for HurI, and ECF sigma factor [64]. The S1P in this system has also not been identified, although a candidate gene approach excluded 4 candidate S1Ps [64].

4.4. Salmonella enterica

RseP, the S2P in *S. enterica s. typhimurium*, plays a role in the *sigE* stress response system that is similar to the *E. coli* DegS/RseA/RseP system. In contrast to *E. coli*, Salmonella *sigE* is not essential *in vitro*,

Table 2
Signaling systems in bacterial pathogens mediated by S2Ps. Listed is the bacterial pathogen, the S1P (if known or required), the substrate of the S2P, and the S2P itself. If the S2P signaling system controls a transcriptional regulator, it is listed along with the activating signal/physiologic function of the pathway.

Pathogen	S1P	Substrate	S2P	Transcriptional regulator	Activating signal/pathway	Ref
Vibrio cholerae	?	TcpP	YaeL	TcpP	Shuts off toxin production in conditions that limit virulence gene expression (Ph 8.5)	[68]
Vibrio cholerae	DegS	RseA	YaeL	SigE	Unfolded OMPs/cell envelope stress response	[68]
Salmonella enterica	DegS	RseA	RseP	SigE	Unfolded OMPs/cell envelope stress response	[67]
Salmonella enterica	none	RseA	RseP	SigE	Acid/acid resistance	[67]
M. tuberculosis	?	RskA, RslA, RsmA, ?	Rip1	SigK, SigL, SigM	Host signals/cell envelope remodeling, catalase-peroxidase, resuscitation promoting factor, growth and persistence in the mouse.	[51,52]
M. tuberculosis	?	PBP3	Rip1	none	Oxidative stress	[53]
Enterococcus faecalis	SPase II	cCF10, cAD1, cPD1	Eep	none	Peptide pheromone production	[70–72,78,79]
Enterococcus faecalis	?	?	Eep	?	?/Virulence in Endocarditis model independent of conjugation function	[75]
Pseudomonas aeruginosa	AlgW	MucA	MucP	AlgU	Mucoid transformation via alginate production	[57,59,61,62]
Pseudomonas aeruginosa	?	FvpR, FoxR, FiuR	MucP	PvdS, FpvI	Siderophore biosynthesis and uptake	[63]
Bordetella bronchiseptica	?	? HurR	HurP	?HurI	Iron uptake	[64]

but plays an essential role in pathogenesis [65,66]. The SigE pathway also has a distinct role in response to acid stress. Acid conditions induce transcription of SigE target genes, a response that is DegS independent but RseP dependent [67]. Similar to the *E. coli* system, heat stress induction of *sigE* requires DegS. Also in contrast to OMP induction of the SigE pathway, RseP lacking its PDZ domain (RsePΔPDZ) constitutively cleaves RseA, but acid stress induction of SigE targets is abolished [67]. This study implies that acid stress directly activates cleavage of RseA at the S2P cleavage step and is independent of a S1P.

4.5. V. cholerae

In contrast to many of the systems discussed thus far, in which a transcriptional regulator is held inactive by a transmembrane anti-Sigma factor, two transmembrane regulators of *V. cholera toxT* transcription, TcpP and ToxR, are active as transcriptional regulators when membrane bound. TcpP is required for the induction of the *toxT* gene, which in turn directly activates cholera toxin (*ctxAB*) and the toxin-coregulated pilus (tcp operon, including TcpA). The TcpP protein is unstable in the absence of another protein TcpH. *Vibrio cholera* YaeL (S2P), the homolog of *E. coli* YaeL/RseP, was identified in a screen for mutants that stabilized TcpP in a *tcpH* null strain [68]. This study also showed that this negative regulation of virulence gene expression by YaeL proteolysis is operative in wild type cells when *V. cholerae* is placed in conditions that turns virulence genes off (pH 8.5, 37°C) [68]. Although there appears to be a site one cleavage event of TcpP that precedes YaeL degradation, DegS was not required for this degradation and the S1P has not yet been identified [68,69]. In contrast, DegS and YaeL appear to both be required for induction of the *V. cholera rpoE* response as judged by the similar sensitivity of the *rpoE*, *yaeL* and *degS* mutants to 3%Ethanol [68], suggesting that YaeL (RseP) has at least two substrates (TcpP and RseA) in *V. cholerae* and both positively and negatively influences gene expression based on proteolysis of either a negative (RseA) or positive(TcpP) regulator.

4.6. Enterococcus faecalis

E. faecalis is a gram positive, naturally competent bacterium that causes urinary tract infections, endocarditis, and infections of indwelling catheters. *E. faecalis* has a pheromone-inducible plasmid transfer system in which plasmid free cells secrete a pheromone called cCF10 (or other similar pheromones such as cAD1 or cPD1), which induces transfer of the pCF10 plasmid from plasmid bearing cells. cCF10 is an octapeptide pheromone that is encoded within the signal sequence of a lipoprotein. After cleavage of the lipoprotein signal peptide by signal peptidase, the membrane embedded signal peptide is further processed by the S2P Eep (for enhanced expression of pheromone) [70–72]. This function of Eep is remarkable because it implicates a S2P in production of an extracellular diffusible signal, and raises interesting questions about whether and how Eep recognizes signal sequences that encode pheromones and differentiates these proteins from other signal sequence remnants [73]. In this regard, the function of Eep is reminiscent of the recently proposed function of *E. coli* RseP and *B. subtilis* RasP in cleaving remnant signal peptides [74]. In addition to its role in pheromone production, Eep was also recently identified in an *in vivo* screen for *E. faecalis* promoters induced in an abscess model [75]. The *eep* promoter was upregulated in this model and a Δ*eep* strain was highly attenuated in an endocarditis model [75]. Importantly, this virulence function of Eep is apparently independent of its function in enhancing plasmid conjugation as this screen was performed in a strain lacking a conjugative plasmid [75]. The Eep substrates mediating the virulence phenotype have not yet been identified.

4.7. Cyanobacteria

Several S2Ps have been identified recently in Cyanobacteria, but their full functions are still being elucidated. The *Synechocystis* S2P Slr0643 is required for acid resistance and may mediate this effect through control of the SigH pathway in that organism [76]. *Anabaena variabilis* encodes five putative S2Ps, all of which can cleave *B. subtilis* pro-SigmaK when co-expressed in *E. coli* [77], but their substrates and physiologic function in *Anabaena* await further study.

5. Summary and common themes in bacterial S2P pathways

Based on the functions of prokaryotic S2Ps presented above, we have derived three major S2P functional groups, which we illustrate in Fig. 1. The first group is the most abundant and illustrated in Fig. 1A. These are the systems similar to the RseP pathway of *E. coli*, which use coupled proteolytic destruction of a transmembrane anti-sigma factor to active a soluble sigma factor. The second group, which is illustrated in Fig. 1B, are systems in which the proteolytic target of the S2P is itself a transmembrane transcription factor. In the case of *B. subtilis* SpoIVFB, the substrate is a transmembrane precursor of SigK, which is inactive when membrane bound. In *V. cholerae* the substrate is TcpP, which is active in the membrane and therefore its proteolysis inhibits transcription. The third group is illustrated in Fig. 1C and consists of systems in which the S2P acts to degrade a membrane protein without a downstream transcriptional function. Examples of these systems include the destruction of $PodJ_S$ by MmpA and maturation of Enterococcal pheromones by Eep from lipoprotein signal peptides. We anticipate that further variations on these themes will continue to emerge as more S2P systems are studied in additional bacterial systems.

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