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Regulation by destruction: design of the σ^E envelope stress response

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The signal transduction pathway governing the σ^E-dependent cell envelope stress response in *Escherichia coli* communicates information from the periplasm to σ^E in the cytoplasm via a regulated proteolytic cascade that results in the destruction of the membrane-bound antisigma factor, RseA, and the release of σ^E to direct transcription. Regulated proteolysis is used for signal transduction in all domains of life, and these pathways bear remarkable similarities in their architecture and the proteases involved. Work with the pathway governing the σ^E response has elucidated key design principles that ensure a rapid yet graded response that is buffered from inappropriate activation. Structural and biochemical studies of the proteases that mediate signal transduction reveal the molecular underpinnings enabling this design.

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Introduction

A major role of signal transduction pathways is to communicate information about the state of a cell or the surrounding environment to the transcriptional machinery to ensure that the proper genes are expressed at the proper time. A fundamental problem in signal transduction encountered by all living systems is the topological constraint posed by a biological membrane. One of the solutions to this problem, found in all kingdoms of life, is to tether a transcription factor to the membrane and control its activity by a proteolytic cascade involving two membrane-bound proteases that act sequentially to release the transcription factor from the membrane in response to an inducing signal. This type of pathway is often called regulated intramembrane proteolysis (RIP) and was first discovered in mammalian cells as a mechanism to coordinate the expression of genes required for cholesterol biosynthesis with the levels of cholesterol in cellular membranes and in Gram-positive bacteria as a mechanism to coordinate forespore and mother cell gene expression during sporulation [1,2]. RIP pathways were subsequently discovered in many other eukaryotic and bacterial systems regulating diverse responses. Some responses, such as intercompartmental stress responses, are controlled by RIP pathways in both bacteria and humans demonstrating the evolutionary success of this pathway design [3–6].

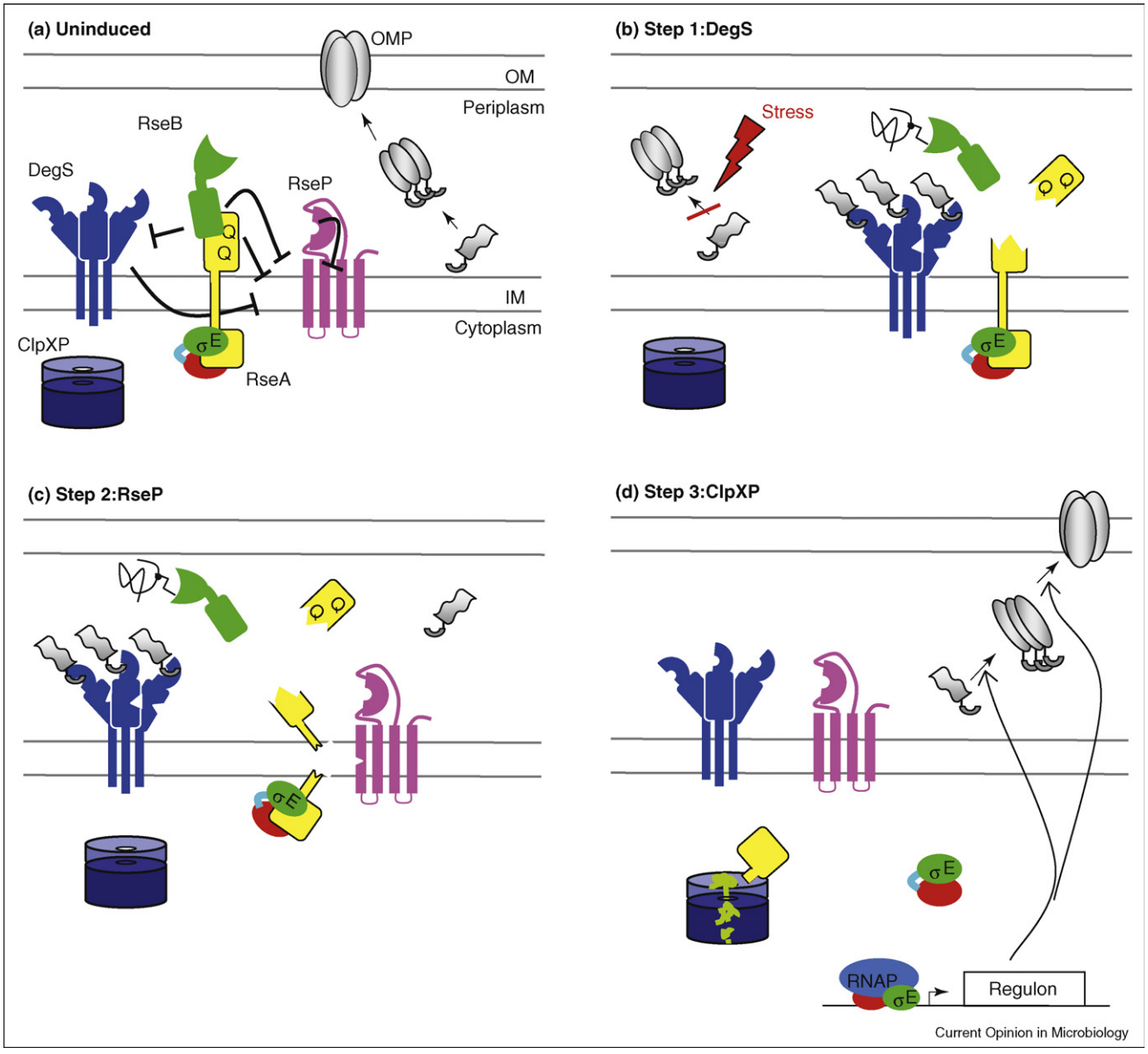
Perhaps the RIP pathway most extensively studied at a molecular level is that controlling the σ^E-dependent cell envelope stress response in *Escherichia coli* [7]. Several key design principles have been elucidated that govern this regulatory pathway [8••]. First, the system can be rapidly activated in response to the inducing signal. Second, the signaling pathway can be activated to different extents, producing a graded response. Third, the system is insulated from stochastic fluctuations that might activate the pathway independently of an inducing signal. Recent biochemical and structural studies reveal the molecular underpinnings of these properties and will be the subject of this review. Although the details of the regulatory interactions that govern other RIP pathways are likely to differ, the overall key design principles uncovered by the work on the σ^E system are likely to be shared.

Regulation of the σ^E-dependent cell envelope stress response in *E. coli*

The key players

The outer membrane of Gram-negative bacteria is crucial for viability; therefore stress responses have evolved in these organisms to maintain its integrity [9]. In *E. coli*, cell envelope stresses that disrupt the folding of outer membrane porins (OMPs) trigger a RIP pathway that results in the activation of the alternative sigma factor, σ^E [3,4,10]. In the absence of inducing signals, σ^E is held at cytoplasmic side of the inner membrane by the antisigma factor RseA, a single-pass membrane protein (Figure 1a) [11,12]. A periplasmic protein, RseB, binds to the periplasmic domain of RseA and enhances the inhibition of σ^E (Figure 1a) [11,12]. Upon cell envelope stress, σ^E is released from RseA by a proteolytic cascade whose end result is the complete degradation of RseA and the release of σ^E to direct transcription (Figure 1) [13]. The RIP proteases of the cascade, DegS and RseP (formerly known as YaeL), act sequentially cleaving RseA first in the periplasmic (Figure 1b) and then in the transmembrane region (Figure 1c) [3,4]. The cytoplasmic domain of RseA ($RseA_{cyto}$) bound to σ^E is then released and

Figure 1



Regulated proteolysis leads to the destruction of RseA and the release of σ^E. **(a)** When the pathway is in the uninduced state, DegS (blue) is inactive and RseB (green) is bound to the periplasmic domain of RseA. DegS, RseB, the glutamine-rich regions of RseA (indicated by Q's), and the PDZ domain of RseP inhibit RseP (magenta). σ^E (red and green) is bound to $RseA_{cyto}$. Outer membrane porins (OMPs) (gray-shaded shapes) enter the periplasm via the Sec secretion machinery (not shown) and are escorted to the outer membrane by a series of chaperones. **(b)** Upon cell envelope stress, OMP folding is disrupted. The C-terminal peptides of unfolded OMPs bind to DegS, activating DegS. RseB is also removed from RseA, perhaps by lipoproteins as diagrammed. DegS cleaves RseA in the periplasmic domain. **(c)** RseP cleaves RseA in the transmembrane region. **(d)** ClpXP (light and dark blue) degrades $RseA_{cyto}$ releasing σ^E to interact with RNA polymerase (RNAP) and direct transcription. σ^E regulon members restore proper OMP folding, resetting the pathway. OM, outer membrane. IM, inner membrane.

degraded by cytoplasmic proteases, primarily ClpXP (Figure 1d) [8••,10,14].

The inducing signal

The proteolytic cascade is induced by a conserved Y*x*F peptide (where *x* is any amino acid) found at the C-terminus of OMPs [15]. This peptide is normally buried and inaccessible in folded porin trimers. When porin folding is disrupted, the peptide is exposed and binds to DegS, activating DegS to cleave RseA and initiate the response [15]. Proper porin folding and transit to the outer membrane involves a series of steps, and disruption of this pathway at any point may lead to improperly folded porins with exposed C-termini. After porin monomers

emerge into the periplasm from the Sec machinery, they interact with a series of periplasmic chaperones that facilitate porin folding and may escort the porin subunits to the outer membrane [16•]. Mature porin trimers are inserted into the outer membrane by a complex that includes both integral membrane proteins and lipoproteins [16•]. As a result, efficient porin folding relies on proper lipoprotein maturation, outer membrane lipid and lipopolysaccharide (LPS) synthesis, and protein folding conditions. It has been proposed that porins are essentially the 'canaries in the coal mine' of the outer membrane, providing a sensitive measure of cell envelope homeostasis [17].

The trigger, activation of DegS

All of the key players in the system, the proteases, RseA, and σ^E, are present under noninducing conditions and held in the 'off' state, poised to rapidly respond to unfolded porins. DegS is not only the sensor, but its cleavage of RseA is also the rate-limiting step of the response [8••]. Therefore, the control of DegS is the critical point of regulation, determining both the rapidity and the extent of activation of σ^E. DegS has been the subject of intensive biochemical and structural studies, which have provided key insights into the mechanisms that underlie DegS function.

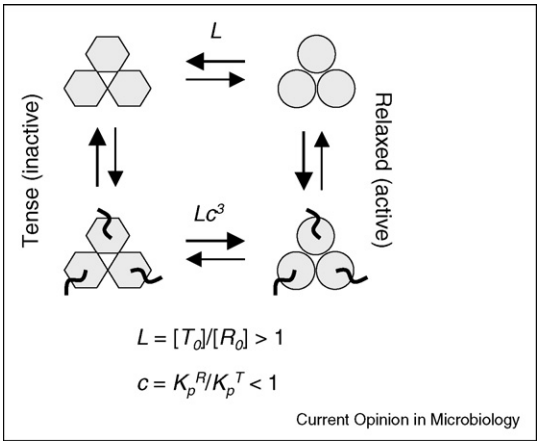
Structural studies

DegS is tethered to the inner membrane by a short N-terminal transmembrane region. The trypsin-like protease and regulatory PDZ domains are in the periplasm, where they can access both the substrate and the inducing signal [18]. The PDZ domain is required to hold DegS in the inactive state [15]. Crystallographic studies of the soluble periplasmic domain of DegS demonstrate the structural basis for regulation [19•,20••,21,22]. The protein forms a funnel-like trimer with the catalytic domains fully accessible at the base of the funnel and the PDZ domains decorating the perimeter [21,22]. In the unliganded form, the active site of DegS is not in the proper conformation for catalysis [21,22]. In the structures of DegS bound to inducing peptide and of a constitutively active variant lacking the PDZ domain, residues in the active site are reoriented so that they are competent for catalysis [19•,20••,21]. Mutational analysis based on the crystal structure suggests that multiple interactions between amino acids in the PDZ domain, the protease domain, and the linker separating the domains stabilize the inactive form of the enzyme. Peptide binding facilitates the rearrangements of several of these interactions both within a subunit and between the subunits that stabilize the active conformation thereby triggering the response ([19•,20••] and J Sohn *et al.*, unpublished).

DegS, an allosteric enzyme

While structural studies provide a snapshot of inactive and active forms of DegS, detailed biochemical analyses have led to a model explaining how interconversion between these two forms of the enzyme yields a system with a sensitive and rapid trigger that can be activated to different extents [20••]. Sohn and coworkers performed biochemical and enzymatic analyses of DegS in the presence of a series of different inducing peptides ([20••] and J Sohn *et al.*, unpublished). They first demonstrated that the degradation of RseA by DegS *in vitro* was sufficiently fast to account for the rapid decrease in RseA levels seen during the initiation of the response *in vivo* [13,23]. They also found that the binding of both Y*x*F peptides and RseA to DegS was positively cooperative and that their experimental data fit well with a computational model based on the classical Monod Wyman Changeux (MWC) concerted model of allostery (Figure 2) [24]. According to this model, an allosteric enzyme such as DegS exists in two states in dynamic equilibrium, the tense state (inactive DegS) and the relaxed state (active DegS). In the absence of ligand, the equilibrium favors the tense/inactive state. Inducing ligands bind to both states, but have a higher affinity for the relaxed/active state and therefore shift the equilibrium in favor of this state. For DegS, both Y*x*F peptides and, to a lesser extent, RseA are inducing ligands. Positive cooperativity arises because ligand binding increases the number of DegS molecules in the active state (Figure 2).

Figure 2



Model of allosteric regulation of DegS by Y*x*F peptides according to the concerted MWC model. Only the fully bound and unbound forms of DegS trimers are shown for simplicity. The tense or inactive form of DegS is indicated by hexagons, and the relaxed or active form of DegS is indicated by circles. Y*x*F activating peptides are represented by curved lines. In the absence of peptide, the ratio of the amount of DegS in the inactive form compared to the active form is given by L and is greater than 1. The affinity of the inactive DegS for peptide (equilibrium dissociation constant $= K_p^T$) is less than that of the active DegS (equilibrium dissociation constant $= K_p^R$). Therefore the ratio of the affinities, c, is less than 1. When DegS is fully bound by peptide, the ratio of the amount of DegS in the inactive form compared to the active form is given by Lc^3 and is less than 1. Therefore, the greater the difference in the affinity of a peptide for active DegS compared to inactive DegS, the smaller the value of c and the larger the concentration of active DegS.

As such, DegS can be maximally activated at a lower concentration of Y*x*F peptides than would be required for a noncooperative enzyme, increasing the sensitivity of the response.

The allosteric model of regulation also provides an explanation for the graded response observed *in vivo*, because the extent of activation of DegS is determined by the difference in affinity of a particular peptide for the active state compared to the inactive state (Figure 2). Sohn *et al.* found that different peptides activated DegS to different extents, suggesting that their relative affinities for the two states varied ([20••] and J Sohn *et al.*, unpublished). This observation also has important implications for the biology of the system. Because sequences outside of the conserved Y*x*F motif, which differ among the porins, appear to determine the extent of activation (J Sohn *et al.*, unpublished and R Chaba *et al.*, unpublished), the identity of the unfolded porin will determine the amount of σ^E released from RseA. Since promoters in the σ^E regulon vary in their affinity for σ^E, the level of activation of the pathway will not only determine the magnitude of the increase in transcription, but also determine which genes in the regulon are transcribed. The response is thus tuned not only to the amount, but also to the identity of unfolded porins.

Inhibition of DegS by RseB

In addition to allosteric regulation of DegS, the proteolytic cascade is also inhibited by the periplasmic protein RseB. RseB binds to the periplasmic domain of RseA and increases its proteolytic stability 2.4-fold [13]. RseB blocks cleavage of RseA by DegS *in vitro*, even when DegS is fully bound by inducing peptide [25••]. These results suggest that an additional step is required for activation of the response, and RseB must first be removed from RseA for DegS to act. Early models proposed that RseB was titrated away from RseA by unfolded proteins; however, recent experiments do not support this model [25••,26,27]. Crystal structures of RseB reveal that it has two domains, one of which closely resembles lipoprotein-binding domains [28•,29•]. This observation suggests that RseB may sense disruptions of lipoprotein synthesis either acting in conjunction with the OMP signals or providing a new trigger for activation of the pathway.

Intramembrane cleavage by RseP

The first cleavage of RseA by DegS releases the C-terminal 67 amino acids from RseA, creating a substrate for the second protease in the pathway, RseP [15]. RseP is a multipass inner membrane metalloprotease with a periplasmic PDZ domain [30]. Both *in vivo* and *in vitro* evidences indicate that RseP is capable of cleaving full-length RseA in the absence of the OMP inducing signal, but does not do so because of a series of inhibitory interactions [27,31,32]. These interactions ensure that RseP cannot degrade RseA independently of the signaling pathway, thereby conferring robustness to the system and safeguarding it against inappropriate activation. RseP is held in the inactive state by DegS, RseB, the PDZ domain of RseP, and two glutamine-rich regions in the periplasmic domain of RseA (Figure 1a) [27,32]. Inhibition by DegS appears to be independent of the other inhibitory interactions and is alleviated when DegS binds to Y*x*F peptides [27]. Once activated, DegS cuts RseA at a site N-terminal to the glutamine-rich regions and the RseB-binding site, presumably physically relieving the remaining inhibitory interactions (Figure 1b) [15,25••]. It is tempting to speculate that the PDZ domain of RseP binds to RseB and/or the glutamine-rich regions of RseA, and these interactions hold RseP in an inactive conformation. However, the molecular mechanisms underlying the regulation of RseP have not yet been elucidated.

Our understanding of the molecular basis of RseP activity is not as advanced as for DegS, which is not surprising given the difficulties associated with biochemical and structural analyses of polytopic membrane proteins. Studies of the cleavage of RseA variants by RseP have localized the cleavage site to the transmembrane domain of RseA raising a major question shared by all such intramembrane proteases [31,33••]. How does hydrolytic peptide bond cleavage occur in the hydrophobic milieu of the membrane? The structure of a closely related protease suggests that intramembrane proteases may have channels within the protein that allow water to access the active site [34•]. The existence of such a channel in RseP is supported by chemical data probing the environment of the active site [35•]. In contrast to DegS, RseP is a relatively nonspecific protease. The major cleavage determinant identified to date is the presence of helix-destabilizing residues in the transmembrane helix [31,33••]. RseP will not only degrade RseA with a heterologous transmembrane domain, but can also degrade model substrates with no sequence similarity to RseA [31]. In other systems the RseP-like protease in the RIP pathway has been shown to have additional substrates [36,37]. Although none have yet been identified for RseP, genetic studies suggest that RseP may have a role in the cell that is distinct from the degradation of RseA [27,38].

Degradation of $RseA_{cyto}$ by cytoplasmic proteases

In many RIP pathways, the transcription factor to be activated is part of the cytoplasmic domain of the membrane-bound substrate, and cleavage by the second protease in the cascade releases the transcription factor from the membrane in a soluble, active form. In contrast, σ^E is tightly bound to the cytoplasmic domain of RseA and additional proteases, primarily ClpXP, are required to release σ^E [8,14,39]. Full-length RseA is not a substrate for ClpXP. However, RseP cleavage releases the

cytoplasmic domain of RseA still bound to σ^E from the membrane, and sequences in the new C-terminus target $RseA_{cyto}$ for degradation by ClpXP [10]. The C-terminal tail of $RseA_{cyto}$ also contains recognition sequences for the SspB adaptor protein, facilitating proteolysis of $RseA_{cyto}$ by ClpXP [14]. Other cytoplasmic ATP-dependent proteases can also degrade $RseA_{cyto}$, ensuring that the system will be fully activated, even if the proteolytic capacity of ClpXP has been exceeded [8••].

Resetting the switch, downregulation of the response

The regulatory pathway is not only designed to have a sensitive trigger specifically tuned to the inducing signal, but also includes a homeostatic mechanism providing a quick and efficient method to reset the switch and deactivate the response. Once σ^E is activated it transcribes the genes in its regulon [40••]. Although the σ^E regulon includes genes that affect many aspects of the cell, a significant fraction of its known regulon members encodes chaperones required for the delivery and assembly of porins in the outer membrane, chaperones required for the delivery and assembly of LPS in the outer membrane, proteases to degrade terminally misfolded porins, and at least two small RNAs that target mRNAs encoding porins for degradation [16•,41–44]. Therefore, the activation of the σ^E pathway increases the capacity of the cell to deliver proteins to the outer membrane, facilitates the removal of misfolded porins, and reduces new porin synthesis reducing the load on the system. Each of these systems helps to lower the level of unfolded porins, thereby reducing the inducing signal and returning DegS to the inactivated state.

Conclusions

Remarkable progress has been made recently uncovering the molecular mechanisms that underlie the regulated proteolytic pathway that controls the σ^E-dependent cell envelope stress response. Several key questions remain unanswered. The role of RseB is still not well understood. How is it removed from RseA so that the proteolytic cascade can start? Although RseB mutants have only minor effects on σ^E activity *in vivo* in *E. coli*, the pathway is fully activated in *Pseudomonas aeruginosa* mutants lacking the RseB homolog suggesting that RseB plays a pivotal role [45]. How is RseP regulated at a molecular level? Potential inhibitors have been identified, but the molecular mechanisms are not known. Are there other substrates for DegS and RseP? Genetic analysis of suppressor mutations suggests that DegS and RseP may have additional roles in the cell beyond regulating σ^E activity [27,38]. How does the proteolytic pathway interact with other pathways that regulate σ^E activity? σ^E is known to be regulated by the alarmone ppGpp independently of DegS and RseP [46•]. Do other regulatory pathways exist? Finally, are the design principles elucidated in the studies described here a hallmark of RIP pathways in other organisms?

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This article describes the first evidence that other regulatory pathways, in addition to the proteolytic cascade, can affect σ^E activity.