Control of the alternative sigma factor σ^E in *Escherichia coli*

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Signal transduction pathways that communicate information from the cell envelope to the cytoplasm of bacteria are crucial to maintain cell envelope homeostasis. In *Escherichia coli*, one of the key pathways that ensures the integrity of the cell envelope during stress and normal growth is controlled by the alternative sigma factor σ^E. Recent studies have elucidated the signal transduction pathway that activates σ^E in response to misfolded outer membrane porins. Unfolded porins trigger the degradation of the σ^E-specific antisigma factor RseA by the sequential action of two inner membrane proteases, leading to release of σ^E from RseA, and induction of the stress response. This mechanism of signal transduction, regulated intramembrane proteolysis, is used in transmembrane signaling pathways from bacteria to humans.

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Abbreviations

Gln	glutamine
LPS	lipopolysaccharide
ppGpp	guanosine-3′,5′-bispyrophosphate
Rip	regulated intramembrane proteolysis
$RseA_{cyto}$	cytoplasmic domain of RseA

Introduction

The bacterial cell envelope is a complex, dynamic compartment that is crucial for the viability of the cell. It ensures the structural integrity of the cell, provides a barrier between the cell and the surrounding milieu, and is the site of important cellular processes including nutrient transport, energy generation, and biosynthesis of many cellular macromolecules [1–4]. The cell envelope is not a static structure but changes in response to environmental stresses and as a result of cell cycle progression [5–7]. In order for the cell to survive, information from the cell envelope must be communicated to the cytoplasm. The crucial components underlying this communication are transmembrane signaling pathways that relay information from the cell envelope to transcription factors in the cytoplasm, generating responses that ensure cell envelope integrity, function and adaptation to cellular needs and environmental conditions.

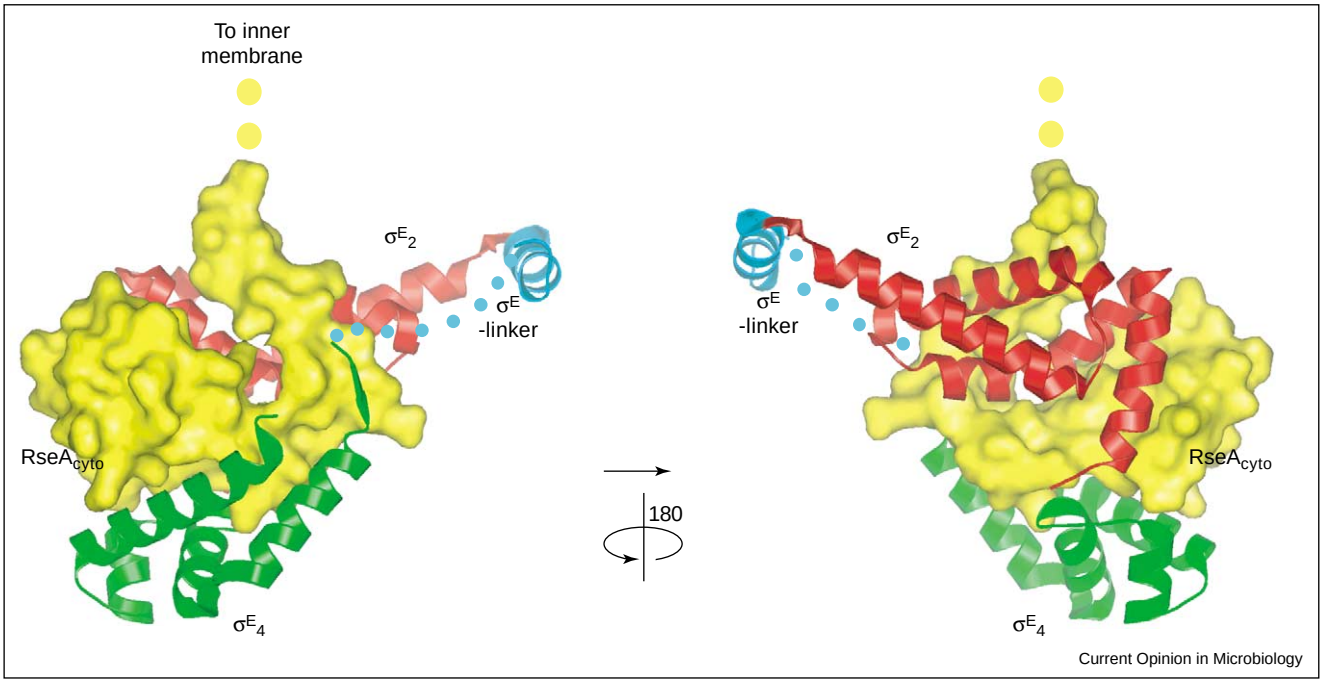
In *Escherichia coli*, the alternative sigma factor σ^E plays a central role in maintaining cell envelope integrity both when the cell envelope is damaged and during normal growth [8]. σ^E controls genes that influence nearly every aspect of the cell envelope and is essential for viability of the bacterium [9•,10,11]. The main focus of research on σ^E has centered on its role and regulation during the stress response. σ^E is induced by stresses that disrupt protein folding in the cell envelope. These stresses include those that affect the entire cell, such as a heat shock and the addition of ethanol, or those that specifically affect the cell envelope such as the overproduction of outer membrane porins or mutations that inactivate periplasmic chaperones [12–17]. On activation, σ^E transcribes the genes in its regulon including those encoding chaperones and proteases targeted to the cell envelope that will refold or degrade misfolded proteins. Despite the intensive work on the role of σ^E during the stress response, the essential function of σ^E is still not understood.

In this review, I focus on new advances in our understanding of the regulation of σ^E. A remarkable picture has emerged in the past few years of nearly the entire signaling pathway that communicates information from the cell envelope to σ^E in the cytoplasm in response to outer membrane porin misfolding. This signal transduction pathway is related to a widely conserved signaling pathway called regulated intramembrane proteolysis or Rip [18]. Rip pathways are controlled by two intramembrane proteases that act sequentially to process a membrane-bound, inactive transcription factor. This processing releases the transcription factor from the membrane, freeing it to activate transcription. Many cellular processes are influenced by Rip including the unfolded protein response governed by ATF6 in higher eukaryotes, the sterol response pathway in mammals, mating pheromone production in *Enterococcus faecalis*, and sporulation in *Bacillus subtilis* [18]. In the case of σ^E, signals from the cell envelope are relayed to σ^E by changes in the proteolytic stability of the inner membrane, antisigma factor RseA [19,20•]. In unstressed cells, RseA binds to σ^E and inhibits its transcriptional activity [21,22,23••]. Upon initiation of the stress response, the inner membrane proteases DegS and YaeL act sequentially to degrade RseA, releasing σ^E [24••,25••].

Inhibition of σ^E by the antisigma factor RseA

The primary point of regulation of σ^E is its interaction with RseA. The cocrystal structure of the cytoplasmic

Figure 1



The cocrystal structure of the σ^E:$RseA_{cyto}$ complex. Two views of the complex are shown; one rotated approximately 180° compared to the other. $RseA_{cyto}$, in yellow, is shown as a molecular surface representation. σ^E, shown as a ribbon diagram, surrounds $RseA_{cyto}$. The σ_2 domain of σ^E is colored red, the σ_4 domain is green and the linker connecting the two domains is cyan. Unstructured regions are represented by dots. The complex is oriented such that the cytoplasmic membrane is at the top of the figure. This figure was generated using PyMOL [43].

domain of RseA, $RseA_{cyto}$, bound to σ^E reveals that RseA inhibits σ^E by sterically blocking its association with RNA polymerase [23••]. σ^E folds into two globular domains with strong structural homology to domains σ_2 and σ_4, of the primary (σ^{70} class) sigma factors [23••]. These domains are essential for promoter recognition and binding to core RNA polymerase [26–28]. $RseA_{cyto}$ forms a single globular domain that is sandwiched between the two domains of σ^E (Figure 1) [23••]. Campbell and coworkers constructed a model of the σ^E-RNA polymerase holoenzyme by superimposing the two domains of σ^E onto the corresponding domains of σ^A in the *Thermus aquaticus* σ^A holoenzyme structure [23••]. The model shows that surfaces on σ^E that contact RseA are also involved in sigma-core interactions, so binding of RseA and RNA polymerase to σ^E is mutually exclusive. *In vitro* biochemical experiments verified that RseA is a competitive inhibitor of σ^E binding to core RNA polymerase [23••]. In fact, σ^E binds to $RseA_{cyto}$ with approximately 300-fold greater affinity than to core RNA polymerase [23••]. This high binding affinity is not surprising in light of the extensive interface between σ^E and $RseA_{cyto}$ and raises the question of how σ^E can ever interact with RNA polymerase *in vivo*.

Because RNA polymerase cannot effectively compete with RseA for binding to σ^E, the σ^E:RseA complex must be disrupted so that σ^E can activate gene expression to support cell viability and to initiate the stress response. The major mechanism employed to release σ^E from RseA is the degradation of RseA [19,20•]. RseA is a relatively unstable protein even in unstressed cells, and continual proteolysis of RseA is essential to provide the cell with sufficient free σ^E for cell viability [24••,25••,29]. During the stress response, changes in σ^E activity are controlled by changes in the degradation rate of RseA [19,20•]. Throughout all phases of the stress response, initiation, adaptation and shutoff, σ^E activity is inversely correlated with the half-life of RseA [19,20•]. On initiation of the stress response, degradation of RseA and σ^E activity increase. When the stress is removed, RseA is dramatically stabilized and σ^E activity decreases. The degradation rate of RseA varies considerably in response to changes in the level of stress in the cell envelope indicating that the proteolysis of RseA is not controlled by a simple on-off switch.

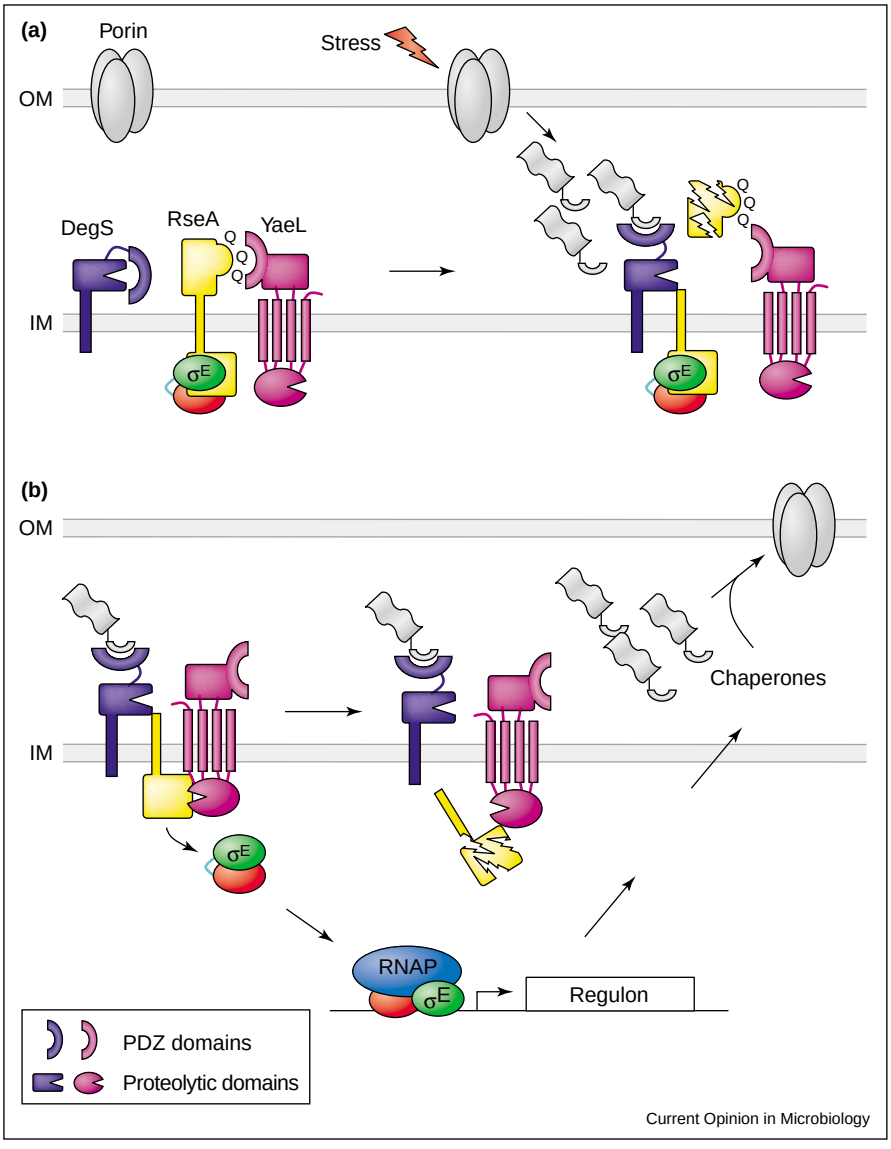
Control of the σ^E-dependent extracytoplasmic stress response by the regulated intramembrane proteolysis of RseA

Proteolysis of RseA is regulated through the activity of the two proteases responsible for its degradation, DegS

and YaeL [24••,25••,29]. DegS and YaeL are inner membrane proteins and fulfill the roles of the Site-1 and Site-2 proteases that act sequentially to cleave their substrate during signaling via Rip [24••,29,30]. The Site-1 protease receives the signal to initiate proteolysis while the Site-2 protease can only cleave the substrate after the Site-1 protease has acted [18]. DegS initiates the proteolytic cascade and cleaves RseA in the periplasmic domain. As soon as the periplasmic domain is removed from RseA by DegS, YaeL degrades RseA further [24••,25••,31••,32••]. Overall, the degradation of RseA is a highly cooperative process as fragments of RseA only accumulate in strains deleted for one of the proteases [24••,25••,31••].

During regulated intramembrane proteolysis, an inducing signal is needed to initiate proteolysis [18]. An inducing signal for DegS is unfolded outer membrane porins [32••].

Figure 2



Model for initiation of σ^E activity in response to unfolded outer membrane porins. RseA and σ^E are colored as in Figure 1, DegS is colored blue and YaeL magenta. **(a)** Initiation of the degradation of RseA by DegS. In unstressed cells, on the left, DegS and YaeL are held in inactive conformations by inhibitory interactions involving their respective PDZ domains. It is not known if the PDZ domain of YaeL directly contacts the Gln-rich blocking region (denoted by Q in the figure) in the periplasmic domain of RseA. However, such an interaction is pictured for simplicity. In stressed cells, on the right, the carboxy termini of unfolded porins bind to the PDZ domain of DegS, and DegS cleaves the periplasmic domain of RseA. **(b)** Release of σ^E from RseA. After removal of the periplasmic domain of RseA, YaeL degrades the remaining membrane-bound fragment of RseA. σ^E is now free to bind to RNA polymerase (RNAP) and transcribe genes in its regulon including chaperones that can refold the porins. IM, inner membrane. OM, outer membrane.

Porins have a complex folding pathway that starts with the translocation of unfolded monomers across the inner membrane and, with the help of several periplasmic chaperones, ends with a folded trimer stably inserted in the outer membrane [33]. It has been known for some time that disruption of outer membrane porin folding is a potent inducer of σ^E [12,34]. Studies of Walsh *et al.* provided insights into the molecular basis of this induction [32••]. DegS possesses a PDZ protein interaction domain, and *in vitro* binding studies revealed that its PDZ domain specifically recognizes a conserved sequence found at the carboxy termini of outer membrane porins [32••]. Degradation of RseA by DegS is activated *in vitro* and σ^E is activated *in vivo* when DegS binds to this peptide. In the absence of the inducing peptide, the PDZ domain inhibits the proteolytic activity of DegS, as deletion of the PDZ domain from DegS leads to activation of σ^E in unstressed cells [32••]. The mechanism of this inhibition is not known, however both the PDZ domain and the active site of DegS are located in the periplasmic region of the protein [29]. The PDZ domain could directly block the active site of DegS, as was observed for the related proteases HtrA2 and DegP [35,36]. Taken together, these data suggest an elegant model to explain how σ^E senses and responds to porin folding problems. The carboxy-terminal peptide of a porin forms part of the trimer interface and is buried in the folded porin trimer [37]. However, if porin folding is disrupted, the peptide is exposed and accessible to DegS. DegS then binds to the peptide via the PDZ domain, alleviating any inhibitory interactions and activating cleavage of RseA (Figure 2a).

Exciting new results fill the remaining gap in our understanding of the pathway, why YaeL requires DegS to degrade RseA [31••]. Two determinants, one in YaeL and one in RseA, prevent YaeL from degrading RseA in the absence of an inducing signal. The periplasmic domain of RseA contains an unusual number of glutamines and the presence of these glutamines makes full-length RseA a poor substrate for YaeL [31••]. On initiation of the stress response, DegS cleaves RseA at residue 148 releasing the glutamine (Gln)-rich inhibitory domains located between residues 162–169 and 190–200 (Figure 2a) [32••]. When the inhibitory domain is gone, YaeL can freely degrade RseA (Figure 2b) [24••,31••]. It is not known how the Gln-rich region blocks degradation by YaeL. However, an interesting mechanism can be envisioned based on the second inhibitory determinant located within YaeL. As with DegS, YaeL contains a PDZ domain that inhibits its protease activity [31••]. However, unlike DegS, the PDZ domain of YaeL cannot act by directly blocking the active site, because it is in the periplasm on the opposite side of the membrane from the active site [30]. An appealing hypothesis is that the PDZ domain of YaeL binds to the Gln-rich regions of RseA either directly or through a third protein, and this interaction holds YaeL in an inactive conformation (Figure 2a). Deletion of either the PDZ domain of YaeL or the Gln-rich region of RseA would then alleviate this inhibition and allow YaeL to degrade RseA in the absence of DegS.

Alternative modes of regulation of σ^E activity

There is strong evidence that additional regulatory mechanisms exist that influence σ^E activity by modulating aspects of the RseA-dependent signaling pathway described here [20•,38]. The regulated proteolysis of RseA is the primary mechanism used to determine the amount of σ^E activity in the cell. However, the relationship between σ^E activity and the rate of degradation of RseA is complex, indicating that other factors may cooperate with RseA and serve to fine-tune the response [20•]. The periplasmic protein, RseB, is one of these factors. Early studies on the regulation of σ^E showed that RseB is a weak inhibitor of σ^E activity and acts by binding to the periplasmic domain of RseA [21,22]. Two roles for RseB in the signaling pathway have been postulated. RseB increases the affinity of RseA for σ^E, and RseB increases the stability of RseA [19,38]. The magnitude of each of these effects is approximately twofold, consistent with the twofold increase in σ^E activity observed in strains lacking RseB [19,21,22,38]. Collinet *et al.* reported that RseB is found in periplasmic inclusion bodies formed by an unfolded variant of the MalE protein, MalE31 [38]. RseB may then act as a sensor of unfolded proteins in the periplasm. As unfolded proteins accumulate, RseB would be titrated away from RseA, decreasing its affinity for σ^E and proteolytic stability. This pathway could then provide the cell with a small boost of σ^E activity under conditions where full activation of σ^E might not be necessary.

Is RseA the only regulator of σ^E activity? The majority of work on σ^E has focused on the stress response, and RseA is clearly the primary regulator of σ^E activity during the response to unfolded proteins. In addition to its role in the stress response, σ^E has recently been shown to play a role in stationary phase [39,40]. In *E. coli*, σ^E activity increases upon entry into stationary phase (A Costanzo and SE Ades, unpublished). The growth-phase dependent activation of σ^E does not require RseA, and instead depends on the alarmone guanosine-3′,5′-bispyrophosphate (ppGpp) (A Costanzo and SE Ades, unpublished). This is the first example of RseA-independent regulation of σ^E activity.

Conclusions

The elegant signal transduction pathway outlined in this review provides a clear picture of how the presence of unfolded porins in the cell envelope is sensed and communicated to σ^E in the cytoplasm to activate the stress response. However, the recent elucidation of the σ^E regulon shows that the role of σ^E in the cell goes beyond

repairing porin folding. In addition to transcribing genes encoding chaperones and proteases, σ^E transcribes genes that can impact many aspects of the cell envelope such as phospholipid and lipopolysaccharide (LPS) biosynthesis, lipoproteins and signal transduction pathways [9•,11]. Walsh *et al*. put forward an intriguing hypothesis that porins act as a global sensor of cell envelope stress [32••]. The proper folding and insertion of porins into the outer membrane requires not only periplasmic chaperones, but also continued biosynthesis and transport of lipopolysaccharides [41]. Alterations in the integrity of the outer membrane can also increase the level of unfolded porins by lowering their incorporation into the membrane [2]. Therefore, an increase in the level of unfolded porins in the cell can signal deficiencies in several aspects of the cell envelope including LPS biosynthesis and the integrity of the outer membrane.

Another important question that remains is whether signals, in addition to unfolded porins, activate the response. Indeed, other inputs almost certainly exist. DegS itself may receive additional signals. Overproduction of porins can induce σ^E, even in a strain that should not be able to sense unfolded porins due to deletion of the PDZ domain of DegS (BM Alba and CA Gross, personal communication). As described above, RseB can modulate the signaling pathway and its input into the stress response is not well understood. Finally, studies of the regulation of YaeL suggest that any interaction that blocks the Gln-rich region in the periplasmic domain of RseA or alters the PDZ domain of YaeL could induce the response independently of DegS.

The contribution of the stress signaling pathway described in this review to the regulation of σ^E in unstressed cells is not clear. Continual degradation of RseA by DegS and YaeL is needed to provide the cell with sufficient free σ^E to support cell viability [24••,25••,29]. However, σ^E can be activated independently of RseA by ppGpp as a culture enters stationary phase (A Costanzo and SE Ades, unpublished). In addition to acting during entry into stationary phase, ppGpp levels rise during starvation for nutrients including amino acids, carbon, nitrogen, or phosphorous [42]. The role of σ^E in nutritional stresses has not been explored. ppGpp may act as a link to coordinate the σ^E-dependent extracytoplasmic stress response with the σ^S-dependent general stress response and other stress responses in the cell during nutrient starvation. Further studies of σ^E will undoubtedly provide new insights into the regulation of this important factor, its role in the maintenance of cell envelope homeostasis, and its integration into global regulatory networks.

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