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Envelope stress responses: An interconnected safety net

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Abstract

The *Escherichia coli* cell envelope is a protective barrier at the frontline of interaction with the environment. Fidelity of envelope biogenesis must be monitored to establish and maintain a contiguous barrier. Indeed, the envelope must also be repaired and modified in response to environmental assaults. Envelope stress responses (ESRs) sense envelope damage or defects and alter the transcriptome to mitigate stress. We will review recent insights into stress sensing mechanisms of the σ^E and Cpx systems and the interaction of these ESRs. Small RNAs (sRNAs) are increasingly prominent regulators of the transcriptional response to stress. These fast-acting regulators also provide avenues for inter-ESR regulation that could be important when cells face multiple contemporaneous stresses, as is the case during infection.

Keywords

Outer membrane; Cpx; σ^E; sRNA

Monitoring the cell envelope

Gram-negative bacteria such as *Escherichia coli* are **diderm** (see Glossary) cells with an outer membrane (OM) that is separated from the inner membrane (IM) by an aqueous periplasmic space which houses the peptidoglycan cell wall [1] (Figure 1). The OM is an asymmetric lipid bilayer where **lipopolysaccharide (LPS)** forms the surface-exposed leaflet and phospholipids form the periplasmic leaflet [2]. While it remains unclear how phospholipids reach the OM, LPS is delivered by the transenvelope Lpt complex [3] (Figure 1). There are two classes of OM proteins: transmembrane **β-barrel proteins (termed OMPs)** [4]; and **lipoproteins** whose acylation anchors them in the bilayer [5]. These hydrophobic proteins require chaperones for transit across the periplasm. For OMPs, the major chaperone in *E. coli* is SurA, with Skp and the chaperone-protease DegP having back-up roles [6]; for lipoproteins, the chaperone is LolA [5]. Chaperones deliver OMPs to the β-barrel assembly machine (Bam) complex for assembly into the OM [4] (Figure 1). Lipoproteins are transferred from LolA to LolB for anchoring into the OM [5] (Figure 1). The envelope protects cells against environmental assaults, the immune system, and

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antibiotics [2]. Given the complexity of envelope biogenesis, several envelope stress responses (ESRs) are tasked with monitoring for defects or damage and restoring homeostasis. For example, the σ^E response is potently activated by OMP assembly defects, while the Cpx **two-component system (TCS)** responds to periplasmic or IM protein misfolding [7,8].

Understanding how signals from multiple stresses are integrated remains an ongoing challenge. Efforts in cataloging ESR **small RNAs (sRNAs)** suggest these molecules may interface between ESRs. Intriguingly, the σ^E and Cpx signaling pathways are highly interconnected. Here, we review recent progress in understanding the stress-sensing mechanisms of these two ESRs, as well as their sRNA effectors. While this review focuses on σ^E and Cpx responses, it is notable that other signal transduction systems also respond to envelope damage and significantly remodel the envelope. Both the RcsBCDF phosphorelay and the PhoPQ TCS sense LPS layer damage inflicted by cationic antimicrobial peptides (CAMPs) and respond by inducing extracellular polysaccharide production and LPS modifying enzymes, respectively, to fortify the OM [9,10]. The EnvZ/OmpR TCS responds to osmotic challenge by regulating major OMP porins [11] .

The σ^E response: a two-signal model for activation

E. coli produces an alternate **σ factor** (σ^E, RpoE) that enables RNA polymerase to increase transcription of genes involved in cell envelope biogenesis [12]. Many of the steps leading to σ^E activation have been described in molecular detail [7] (Figure 2). Under non-inducing conditions, the IM anti-σ factor RseA sequesters σ^E to the plasma membrane [13]. The presence of unfolded OMPs in the periplasm is the most thoroughly characterized signal for σ^E activation [14,15]. Signal sensing triggers a cascade of regulated proteolysis steps that degrade RseA and release σ^E into the cytosol where it can promote transcription [7]. σ^E-dependent promoters increase production of periplasmic OMP chaperones, the Bam complex, and other factors required for OM homeostasis [12]. The negative regulatory arm of the response relies on sRNAs that prevent OMPs synthesis until stress is relieved (discussed later).

Unfolded OMPs are sensed by DegS, an IM serine protease [16]. The periplasmic PDZ domain of DegS maintains the protease in an inactive state [14]; this inhibition is relieved when the PDZ domain binds peptides corresponding to C-terminal residues of OMPs [14]. Such sequences are inaccessible in properly folded OMPs, but they accumulate in the periplasm if OMP folding is defective [13]. Peptide binding by DegS reorients the active site to facilitate catalysis [17]. Two consecutive proteolytic cleavages of RseA are required to release σ^E. DegS removes a periplasmic domain of RseA and this cleavage is the rate-limiting step of σ^E activation [18].

Following degradation by DegS, the remaining RseA protein becomes sensitive to proteolysis by RseP, a zinc metalloprotease belonging to the S2P group of intra-membrane proteases (RIPs) [19]. The periplasmic domain of full length RseA acts as a size exclusion filter that prevents cleavage of the protein by RseP. [20]. The RseP active site is within the IM bilayer; recent insights demonstrate how an unusual RseP intramembrane β-strand

unfolds the RseA target site to make it amenable for proteolysis [20]. Degradation by RseP releases a soluble fragment of RseA—still clutching σ^E—into the cytosol [21]. Cytoplasmic proteases, most notably ClpXP, subsequently liberate σ^E [21].

A periplasmic protein, RseB, negatively regulates σ^E activation by binding RseA and preventing cleavage by DegS [7]. *In vivo*, discreet OMP peptides (with sequences N-terminal to the DegS-activating peptide) can displace RseB from RseA; but, these peptides were insufficient in an *in vitro* reconstituted system and an additional RseB signal was hypothesized [15]. Recent *in vitro* work proposes the acyl chains of LPS bind RseB and are the signal that displaces it from RseA [22]. *In vivo*, mutations that either reduce LPS acylation or truncate the LPS core polysaccharide induce σ^E [22]. Moreover, a folding-defective variant of LptD (the OMP that inserts LPS into the OM [3]) induces σ^E even in the absence of the OMP-sensing DegS PDZ domain, suggesting LPS transport defects are sensed [22]. A two-signal hypothesis for σ^E activation is currently proposed: full activation requires sensing of both unfolded OMPs (to activate DegS) and periplasmic LPS (to displace RseB from RseA) [22].

A lipophilic signal for RseB had been anticipated given the protein's structural similarity to LolA and LolB, the proteins involved in OM lipoprotein transport and insertion, respectively [15,23,24]. Yet, the most potent σ^E-activating mutations truncate the LPS core polysaccharide, which is distal to the lipophilic LPS components [22]. What conditions might cause production of truncated, σ^E-inducing LPS molecules in vivo remains an unresolved question. In wildtype cells, the lipid and core components are produced stoichiometrically and yield a consistent LPS structure [25]. One suggested possibility is that truncated LPS might be poorly transported by the Lpt transenvelope complex, causing it to accumulate in the periplasm [22]. σ^E induction by an LptD mutant seems to support this model [22]. However, evidence that LPS can accumulate in the periplasm in a manner that exposes its acyl chains to RseB is lacking. LPS is extremely lipophilic and poorly soluble; indeed, the Lpt bridge (Figure 1) is needed to provide a conduit for the lipophilic moieties as they pass through the aqueous periplasm [26]. The bridge is only formed when the OM Lpt components are capable of receiving LPS [27]. Depletion of Lpt proteins causes LPS accumulation within the IM and results in abnormal membrane structures [28,29]. It seems unlikely that LPS is excreted directly into the periplasm. How, then, might RseB encounter LPS acyl chains? Perhaps some accessory protein can receive LPS from defective Lpt machinery and transfer the molecule to RseB, akin to the lipoprotein transfer that occurs between LolA and LolB [30]. Clearly, a complete understanding of the RseB signal remains an important challenge.

Stress sensing by the Cpx response

The core of the Cpx ESR relies on a canonical TCS: CpxA is the IM sensory histidine kinase; CpxR is the DNA-binding response-regulator (Figure 2). Cpx responds to a broad set of conditions that include elevated pH, high salt concentrations, and alterations in IM lipid composition [8]. Recent work also suggest Cpx plays a role in sensing and responding to peptidoglycan cell wall defects [31-33]. One common theme among inducing conditions is that they cause protein misfolding. Sensing is nonetheless specific and misfolded OMPs fail

to activate Cpx [34]. Misfolded IM and periplasmic proteins, and defects in protein translocation across the IM are all sensed by Cpx [8]. Increasingly, it appears that Cpx is primarily tasked with defending IM integrity [8].

Existing evidence suggests that CpxA directly senses stress [35,36]. CpxA consists of two transmembrane domains with a periplasmic loop that acts as a sensory domain [36]. CpxA activation leads to autophosphorylation of its cytoplasmic histidine kinase domain, which allows for phosphotransfer to the receiver domain of CpxR [36]. Phosphorylated CpxR binds cognate DNA sequences to regulate gene expression [36]. CpxA has both kinase and phosphatase activity, enabling it to rapidly control the extent of CpxR phosphorylation (and hence the strength of the response) [36].

A recent structure of the *Vibrio parahaemolyticus* CpxA sensory domain shows it forms a **PAS domain** of five β-strands and three α-helices [37]. Mutations in the sensory domain can activate CpxA. For example, the well-studied *cpxA24* mutation deletes 32 residues and entirely removes a C-terminal sensory domain α-helix [36,37]. CpxA24 is constitutively activated and signal blind, suggesting that disrupting proper sensor domain folding may directly trigger CpxA kinase activity [36,38].

Two auxiliary signaling proteins modulate CpxA activation: the positive regulator NlpE and the negative regulator CpxP [39,40]. NlpE is an OM lipoprotein that activates Cpx upon *E. coli* adhesion to abiotic surfaces; such signaling is important since both NlpE and the Cpx response are required for efficient adhesion to hydrophobic surfaces [41]. Unfolded NlpE is proposed to directly contact CpxA from the OM to induce signaling [42]. How cell surface adhesion might unfold NlpE in the periplasm is not yet clear.

Among the most upregulated genes of the Cpx response is *cpxP* [43]. The periplasmic CpxP protein completes a negative-feedback loop by inhibiting CpxA kinase activation [44]. Based on its periplasmic localization, CpxP is suggested to directly block the CpxA sensory domain to inhibit signaling [44]. However, detecting direct CpxP-CpxA interaction has proven challenging [8,37,45]. CpxP forms a dimer with a large charged surface which is proposed to mediate electrostatic interaction with CpxA [45,46]. Indeed, high salt concentrations seem to displace CpxP [45]. Mutations that alter the CpxP surface impair CpxA inhibition and—for at least the D61 residue—even conservative substitutions are not tolerated, hinting at specific biochemical interactions [45-47]. Recently, CpxP-CpxA cross-linking and affinity purifications *in vivo* have provided evidence supporting direct interaction [48]. But, as CpxP levels in wildtype cells are extremely low, these interaction studies rely on overexpression of both proteins above their physiological levels [48]. Detecting a dynamic interaction between CpxA and CpxP at native levels remains an ongoing challenge.

CpxP has weak chaperone activity that is important for its role in clearing misfolded P pilus subunits from the periplasm [49]. CpxP is titrated by misfolded pilins and it delivers them to the periplasmic protease DegP so that both CpxP and its cargo are degraded [49]. Displacement of CpxP from CpxA is not itself a mechanism for sensing. In fact, CpxA is activated by alkaline pH and misfolded pilins even in the absence of CpxP [38]. The

regulatory and effector functions of CpxP must be important during to the Cpx response since production of the *cpxP* mRNA transcript is so highly induced. Astonishingly, that abundant transcript also encodes for another Cpx effector, an sRNA that has only recently been discovered.

Small RNAs packed with big responsibilities

sRNAs are deployed by each of the major ESRs. sRNAs act as important regulatory molecules that can rapidly alter gene expression profiles. Typically, sRNAs bind multiple target mRNA transcripts and act negatively to either prevent translation or promote mRNA degradation, or both [50]. Some sRNAs do act positively on target mRNAs, for instance by relieving RNA structures that inhibit translation [51]. The general RNA chaperone Hfq binds to sRNAs to stabilize them, aid in target binding, and promote recruitment of RNA degradation machinery [50]. A number of new sRNAs have been identified as effectors for each of the ESRs.

Cpx and σ^E sRNAs prevent OMP synthesis

By virtue of being a component of the RNA polymerase, σ^E can activate transcription but is unable to directly repress gene expression (though a recent example does illustrate that a σ factor can increase transcription of an overlapping non-coding RNA as a means of preventing gene expression [52]). Nonetheless, σ^E activation causes a marked decrease in OMP levels [53]. Strong σ^E-dependent promoters produce the sRNAs MicA and RybB that reduce OMP synthesis. MicA regulates both *ompA* and *lamB* production [54-56]. RybB regulates *ompC* and *ompX* expression [57]. The importance of reducing OMP levels under stress conditions is underscored by convergent targeting of *ompA*, *lamB*, *ompW*, and *tsx* transcripts by both MicA and RybB at non-overlapping sites of the mRNA [58]. With regards to envelope stress, the old adage from Will Rogers applies: “If you find yourself in a hole, stop digging.” Remarkably, MicA also provides an interface between stress responses by directly regulating the *phoPQ* transcript that encodes the PhoPQ TCS responsible for modifying LPS under certain stress conditions [56,58]. Additional, non-OMP MicA and RybB targets have been identified, but their contribution to σ^E responses remains to be characterized [58].

sRNA components of the Cpx response have only recently been recognized [59,60]. CpxR increases levels of the sRNAs OmrA, OmrB and MicF, which all belong to the EnvZ/OmpR TCS regulon [61,62]. Cpx induces these sRNAs by producing an IM protein, MzrA, that then directly stimulates the EnvZ histidine kinase and so connects the Cpx response to EnvZ/OmpR [63,64]. Indeed, OmrA/B is known to be induced by Cpx via MzrA, and it is presumed (though untested) that MicF is similarly regulated [63].

OmrA/B redundantly target several OMP-encoding transcripts for degradation (including *cirA*, *fecA*, *fepA*, and *ompT*) [65]. MicF negatively regulates the major porin OmpF [62]. Hence, the Cpx response engages EnvZ-OmpR and its sRNAs to further downregulate OMP production. The sRNAs also regulate signaling: OmrA/B target the *ompR-envZ* transcript in a negative-feedback loop [65]; while MicF targets the *cpxRA* transcript to lower CpxR and CpxA levels [60,66]. Curiously, while MicF lowers CpxR levels it does not reduce

transcription from strong CpxR-dependent promoters [60]. An intriguing hypothesis is that MicF disproportionately affects weaker CpxR promoters to sculpt the extent of the regulon invoked during stress [60]. In any case, MicF is remarkable for establishing a negative-feedback loop that is wired through two signal transduction systems.

RprA: an sRNA of the Rcs and Cpx responses

The RprA sRNA is a highly induced component of the Rcs response (Box 1) [67]. RprA promotes production of σ^S, the master regulator of general stress, by relieving an inhibitory structure in *rpoS* mRNA that hinders translation [51,68]. A key role for σ^S is to promote the transition from planktonic growth to a program of biofilm development [69]. The transcription factor CsgD is central to this transition since it promotes production of cellulose and **curli fimbrae**. σ^S directly increases *csgD* expression but also acts indirectly by upregulating the diguanylate cyclase YdaM (DgcM) whose c-di-GMP production stimulates *csgD* transcription [70]. The RprA sRNA plays an important regulatory role in this biofilm circuit. On the one hand, RprA promotes σ^S production but it impedes production of both YdaM and CsgD [71]. This curious regulatory arrangement is suggested to allow cells to switch off the massive production and secretion of cellulose and curli if envelope defects are detected [69]. The *csgD* mRNA transcript is an amazingly complex nexus for regulatory inputs from as many as six sRNAs, including ESR regulated OmrA/B and RprA [72]. Moreover, *csgD* expression is not only regulated by both σ^S and Rcs (through RprA), but is also directly repressed directly by CpxR and activated by the osmolarity-sensing EnvZ/OmpR system [73,74].

CpxR was recently shown to bind the *rprA* promoter and increase production of the sRNA [60]. RprA overexpression establishes a negative-feedback loop that reduces transcription of strong CpxR-dependent promoters [60]. Hence, it seems RprA induction could allow the Rcs response to potently regulate any output of the Cpx response. It remains unclear how RprA achieves Cpx feedback, though it clearly requires CpxR [60]. A tantalizing possibility is that RprA regulates an undiscovered auxiliary protein capable of modulating CpxR activity. Such proteins are known in other signaling circuits. For example, CheZ dephosphorylates the chemotaxis response regulator CheY [75]; and TorI binds the TorR response regulator to inhibit recruitment of RNA polymerase to promoters [76].

New ESR sRNAs derived from transcript 3’ untranslated regions (UTRs)

A recent breakthrough investigation of RNAs bound to Hfq discovered several new sRNAs that originated from the 3’ UTR of transcripts, revealing an overlooked source of sRNAs [77]. Indeed, a new Cpx response sRNA, CpxQ, was found to originate from the 3’ UTR of a highly induced mRNA transcript [78,79] (Figure 3). In a separate study, a new σ^E sRNA, MicL-S, was found encoded in the 3’ UTR of an annotated gene, though in this case the sRNA is transcribed from a dedicated promoter within that gene's coding sequence and then further processed [80] (Figure 3).

A recent examination of the transcriptomic response to σ^E overproduction identified MicL as the third sRNA of this ESR [80]. MicL and its dedicated σ^E-dependent promoter are encoded entirely in the 3’ region of the gene annotated as *cutC*. MicL is processed into a

smaller product (MicL-S), though the mechanism involved is unknown [80]. In any case, both MicL and MicL-S are unique in having only a single mRNA target: the *lpp* transcript, preventing its translation and promoting its turnover [80]. MicL now accounts for the longstanding observation of reduced *lpp* mRNA levels during σ^E overproduction [12]. Lpp is an OM lipoprotein that forms covalent linkages between the OM and the PG cell wall and is the most abundant protein produced by *E. coli* [81]. Lpp plays no role in OMP or LPS biogenesis, so it is perhaps puzzling why σ^E deploys MicL as an effector. One proposal is that reducing Lpp production may ease demand on the Lol pathway that delivers lipoproteins to the OM; in doing so, MicL could increase Lol pathway capacity for delivering lipoproteins such as BamD and LptE that do have essential roles in OMP and LPS biogenesis, respectively (Figure 1) [80]. Lpp belongs to a growing cohort of "surface-exposed" lipoproteins, since a population of Lpp is detectable outside the cell [82]. The Bam complex has been implicated in surface-exposure of several lipoproteins and is currently the only mechanism described in *E. coli* for lipoprotein translocation to the surface [83-86]. Although Lpp surface-exposure is yet to be directly demonstrated as being Bam-dependent, the prospect raises a curious alternate hypothesis for MicL regulation: perhaps MicL reduces Lpp synthesis to decrease demand on the Bam complex for lipoprotein translocation and allow it to become more dedicated to OMP folding, a process that σ^E monitors intently. Given the homology of RseB to Lol pathway proteins, MicL provides another fascinating link between the σ^E response and lipoproteins that awaits further exploration.

CpxQ is a product of the 3' UTR of *cpxP* mRNA and must therefore be highly abundant during Cpx stress [43,78]. Unlike MicL, CpxQ does not have its own promoter [78]. Rather, normal *cpxP* mRNA decay liberates CpxQ which is stabilized against degradation by Hfq [78]. In *Salmonella*, CpxQ production does not affect CpxP levels [78]. But, in *E. coli*, the presence of CpxQ in the 3' UTR causes a reduction in cellular CpxP levels [79]. CpxQ negatively regulates several targets in *trans*, most notably NhaB (an IM sodium-proton antiporter) and the periplasmic chaperone Skp [78,79]. Despite their distinct localizations, NhaB and Skp appear to be regulated by CpxQ for the same purpose: to protect the **proton-motive force** that is maintained across the IM, which is a source of cellular energy in Gram-negative bacteria [78,79].. NhaB overexpression permeates the IM to protons and its downregulation by CpxQ protects the cell from chemical agents that disrupt the PMF [78]. Skp can challenge the PMF by mislocalizing OMPs into the IM, as discussed later. Multiple Cpx effectors seem to protect the PMF. Another Cpx sRNA, CyaR, acts in a positive-feedback loop to overproduce YqaE, a CpxR-induced IM protein with homology to eukaryotic proteins that modulate membrane potential [60]. The *cpxPQ* RNA transcript specifies two stress effectors directed to different cellular compartments. Despite a common origin, CpxP and CpxQ appear to have distinct roles in combatting stress since there is no condition identified to-date that requires both effectors [78,79]. sRNAs typically fine-tune transcriptional responses; how sRNAs influence the fitness of cells experiencing stress has been better assessed for some sRNAs than others. A more complete appreciation of how newly identified sRNAs contribute to alleviating stress is an ongoing goal.

A hierarchical Cpx-σ^E regulatory axis

A key question of ESR signal transduction pathways is how they interact with one another. With further characterization of the Cpx sRNAs, it is notable that Cpx and σ^E share multiple linkages for inter-ESR communication (Figure 4). Whereas the σ^E response aims to restore OMP folding by inducing chaperone production to maintain nascent OMPs in folding-competent states and by increasing expression of Bam machinery, the Cpx response appears to act antagonistically to σ^E at several points. For example: CpxR represses the *rpoE-rseA-rseB* operon to prevent σ^E production [43,87]; Cpx directly represses major OMP production; deploys sRNAs to further inhibit OMP synthesis [63,88]; and CpxQ inhibits production of the σ^E-induced chaperone Skp [78,79].

Several findings hint that Cpx may function as a failsafe mechanism to protect against prolonged σ^E responses. While the Bam complex catalyzes OMP folding *in vivo*, direct folding of OMPs into membranes *in vitro* is well established [89]. Skp, unlike the major chaperone SurA, can promote direct OMP insertion into membranes [90,91]. Defects in the primary SurA-Bam OMP pathway activate σ^E in order to maintain OMPs in folding-competent states within the periplasm, including by up-regulating Skp [53]. From the periplasm, Skp could assist direct OMP insertion into the OM or could mislocalize OMPs into the IM. OMP mislocalization introduces an ion permeable pore through the IM and is toxic since it collapses the PMF [92]. Indeed, tethering an OMP to the IM results in Skp-dependent toxicity that bears the hallmarks of PMF depletion [79]. The Cpx response combats OMP mislocalization by inhibiting Skp synthesis via CpxQ [78,79].

An OMP "β-sequence" has been proposed to mark nascent proteins in the periplasm as Bam complex substrates that require β-barrel folding [93]. Additionally, OMPs with mutated C termini are poor substrates for Bam, but retain their inherent ability to directly fold into membranes [89]. These OMP mutants might be more likely to attempt direct membrane insertion because they fail to effectively engage Bam. Expressing such OMP mutants *in vivo* potently activates Cpx responses, despite Cpx not being sensitive to misfolded OMP cues [94].

In all, the Cpx-σ^E regulatory axis appears designed to allow initial attempts at OMP assembly recovery via the σ^E response. However, in an ultimate effort to protect the IM and the energy generating capability of the cell, Cpx is engaged to halt σ^E responses, prevent OMP folding, and—by overproducing the periplasmic chaperone-protease DegP—degrade remaining unfolded or misfolded OMPs.

Concluding Remarks

Simple models of stress have been invaluable in identifying ESRs and their effectors. Nonetheless, even in the most thoroughly characterized ESRs, questions remain about how stress is sensed (see Outstanding Questions). During transitions between ecological niches or during infection, bacteria encounter continuous, complex environmental changes that could trigger multiple stress inputs and it remains to be seen how cells interpret these complex signals to mount a coherent response against this onslaught. The ESR sRNAs have

clear functions in regulating effectors as well as feedback functions in regulating responses. Notably, the sRNAs appear to provide numerous avenues for inter-ESR signaling, including by controlling transcripts specifying proteins at the apex of stress signaling circuits (Figure 4). We suggest that sRNAs will prove to play important roles in coordinating the highly interconnected stress responsive network.

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Glossary

Diderm
Cells that enveloped by two membranes

β-barrel proteins (termed OMPs)
A transmembrane OM protein that is formed by wrapping a series of β-sheets into a cylindrical structure. The lumen of the cylinder of OMPs that function as porins forms an aqueous pore through the OM bilayer that allows diffusion of hydrophilic molecules, including nutrients, into the cell

Lipopolysaccharide (LPS).
A glycolipid produced by Gram-negative bacteria consisting of a hexa-acylated diglucosamine to which a series of sugars are sequentially added. These additional sugars are collectively termed the LPS core polysaccharide

Lipoprotein
A protein that is triacylated in the IM at an invariant cysteine residue. Lipoproteins destined for the OM are marked by the absence of an aspartate residue adjacent to the cysteine, which permits their entry into the Lol pathway

Two component system (TCS)
A common bacterial signal transduction system consisting of an IM histidine kinase that detects sensory input and phosphorylates a cognate DNA-binding response regulator. Many TCSs control gene expression

Small RNAs (sRNAs)
Short RNA products that base-pair with multiple target mRNA transcripts in order to regulate their stability or translation

PAS domain
A signal sensory domain that is common in signaling proteins throughout bacteria, archea, and eukaryotes

Curli fimbrae
Amyloid fibrils of curli protein produced and secreted by *E. coli*

Proton motive force (PMF)

A gradient of H^+ concentration across the IM, with a higher H^+ concentration in the periplasm in comparison to the cytosol. The gradient is maintained to establish an electrical potential across the IM. Regulated movement of H^+ into the cytosol provides energy to a number of cellular processes, including generation of ATP

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BOX 1 The Rcs ESR is a complex TCS phosphorelay system

At the core of the Rcs system is the IM histidine kinase RcsC and response regulator RcsB. Stress activates the RcsC kinase activity to ultimately phosphorylate the IM phosphotransferase RcsD which then passes the phosphate to RcsB [67]. Part of the Rcs regulon is controlled by RcsB directly, but regulation of some genes additionally requires the auxiliary transcription factor RcsA [95]. The IM protein IgaA is a negative regulator that inhibits RcsC activation [96]. Well characterized Rcs inducing cues include damage to the LPS cell surface layer caused by cationic antimicrobial peptides (cAMPs) and peptidoglycan cell wall biogenesis defects [97,98]. The OM lipoprotein RcsF is the sensor of these stresses at the cell surface and in the periplasm [9,84,86]. It is proposed that RcsF stress sensing allows the protein to interact with IgaA and thereby relieve inhibition of RcsC, initiating the signaling cascade. A major outcome of the Rcs response is overproduction of the exopolysaccharide colanic acid (which aids in resisting cAMPs) and the system also acts to represses flagella production. Differential regulation of colanic acid and flagella is important for Rcs to promote biofilm maturation [67].

Outstanding questions

- What is the nature of the LPS cue that binds RseB and activates σ^E response? Are σ^E-inducing mutant LPS forms poorly transported to the OM? Do transport defects lead to LPS excretion directly into the periplasm? Does RseB receive LPS directly, or is the glycolipid transferred from an auxiliary protein?
- Can the interaction between CpxA and CpxP be further defined? How does CpxP inhibit CpxA activation? How can CpxA detect such a diverse range of stress conditions?
- Does MicL regulate Lpp to prioritize Lol pathway transport of essential lipoproteins? Is Lpp regulation important during σ^E-inducing stress conditions?
- How do MicF and RprA sRNAs fulfil negative feedback on the Cpx response?
- Does Cpx protect the IM from OMP mislocalization? How are such events sensed?
- How are activating signals from multiple activated ESRs integrated into a protective output response?
- The ESR sRNA network provides extensive interfacing between stress systems, are these connections important during complex stress or during infection?

Trends box

- Bacterial cell envelope biogenesis and integrity is continuously monitored by dedicated stress responses.
- In *Escherichia coli*, the σ^E and Cpx responses maintain homeostasis of the outer membrane and the inner membrane, respectively.
- A proposed lipid signal for σ^E activation has expanded the sensing repertoire of this response.
- sRNAs have become prominent regulators of stress responses, providing effector functions as well as interfacing with other signal transduction systems.
- The σ^E and Cpx signalling exhibits extensive signalling linkages and appear to be antagonistic, perhaps because the cell prioritizes protecting the energy generating functions of the inner membrane.

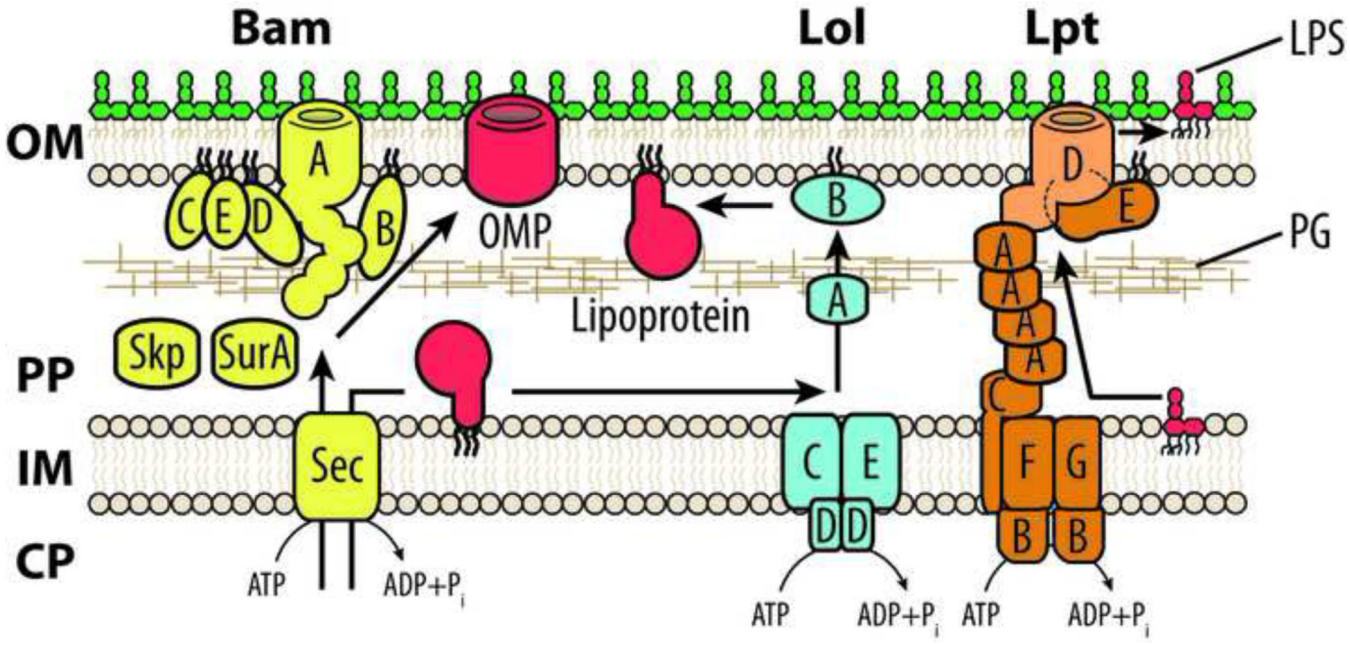

Figure 1. Cell envelope structure and OM biogenesis machines

OMPs synthesized in the cytoplasm (CP) are secreted (via the Sec translocase) into the periplasm (PP) where chaperones such as SurA and Skp prevent their misfolding. The Bam complex (BamABCDE) receives OMPs from chaperones before folding them and facilitating their insertion into the OM. Lipoproteins are also secreted but enter the Lol pathway that extracts the acylated lipoproteins from the IM (via LolCDE) and transfers them to the LolA chaperone. LolB receives lipoproteins at the OM from LolA and anchors them into the bilayer. LPS is transported across the PP by a transenvelope bridge formed by the Lpt complex. LPS is synthesized in the IM before being extracted from the bilayer by the LptBBFG ABC transporter and transferred to LptC. Sequential rounds of ATP hydrolysis move LPS from LptC onto and across the bridge formed by a polymer of LptA. At the OM, the OMP LptD and the lipoprotein LptE act in concert to insert LPS into the outer leaflet of the OM. The PG cell wall resides in the PP. A Substrate OMP for the Bam complex, a substrate lipoprotein for the Lol pathway, and a substrate LPS molecule are colored in red.

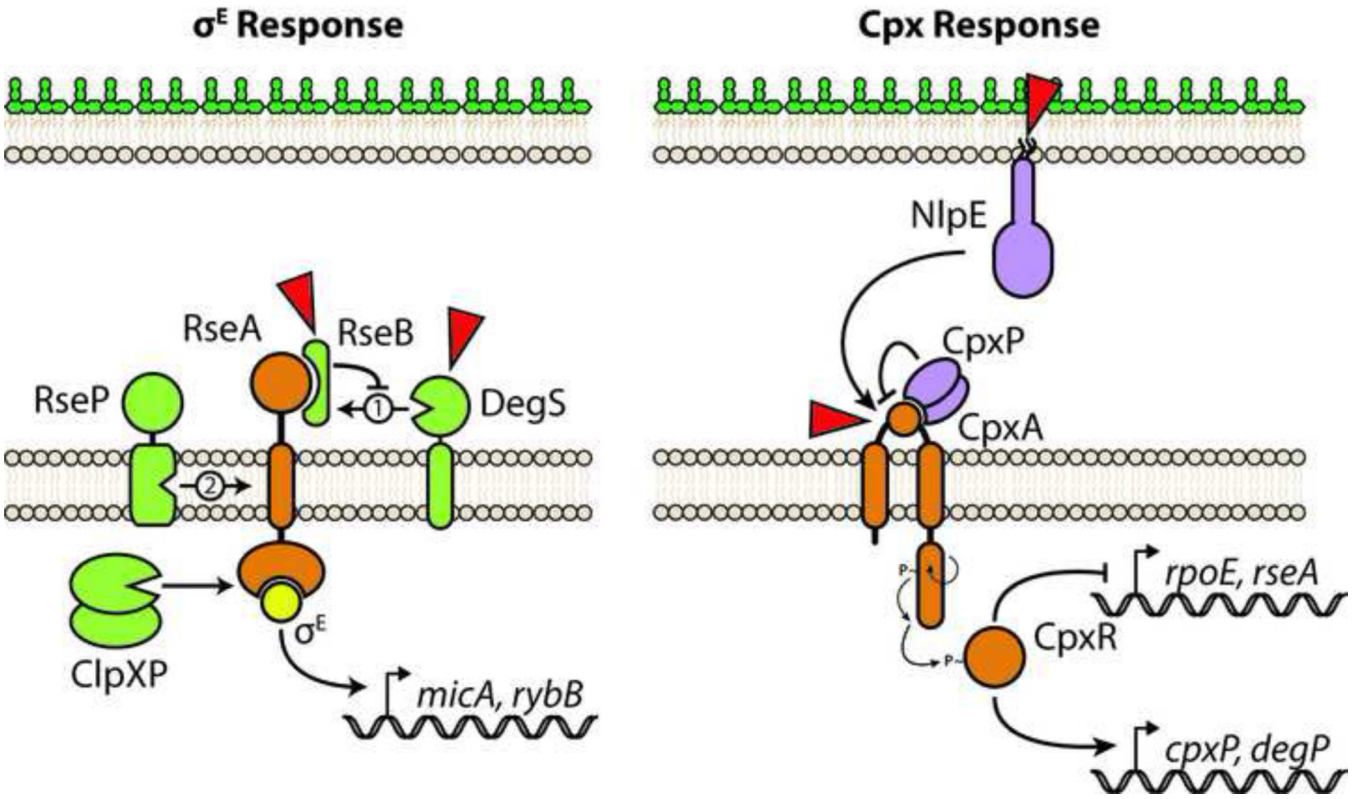

Figure 2. The σ^E and Cpx envelope stress responses of *E. coli*

Left, the σ^E response is activated by a series of proteolysis steps that degrade the anti-σ factor RseA and liberate σ^E into the cytosol where it can increase target gene transcription. The RseA periplasmic domain is first cleaved by DegS. RseB binds RseA and inhibits DegS activity. DegS senses unfolded OMP cues. RseB is proposed to be displaced from RseA in response to LPS binding. RseB displacement allows DegS to cleave RseA and initiate the response. RseA is subsequently cleaved by the intramembrane protease RseP. A cytoplasmic portion of RseA is then degraded by the ClpXP protease to release σ^E. Right, the Cpx TCS responds to numerous signals detected by the periplasmic sensory domain of CpxA. CpxP inhibits CpxA activation likely by interacting with the sensory domain directly. NlpE can transduce cell adhesion signals across the envelope to activate the Cpx response. Stimulated CpxA histidine kinase autophosphorylates and transfers the phosphate to the CpxR response regulator, which alters gene expression. Sensory inputs are illustrated by red triangles.

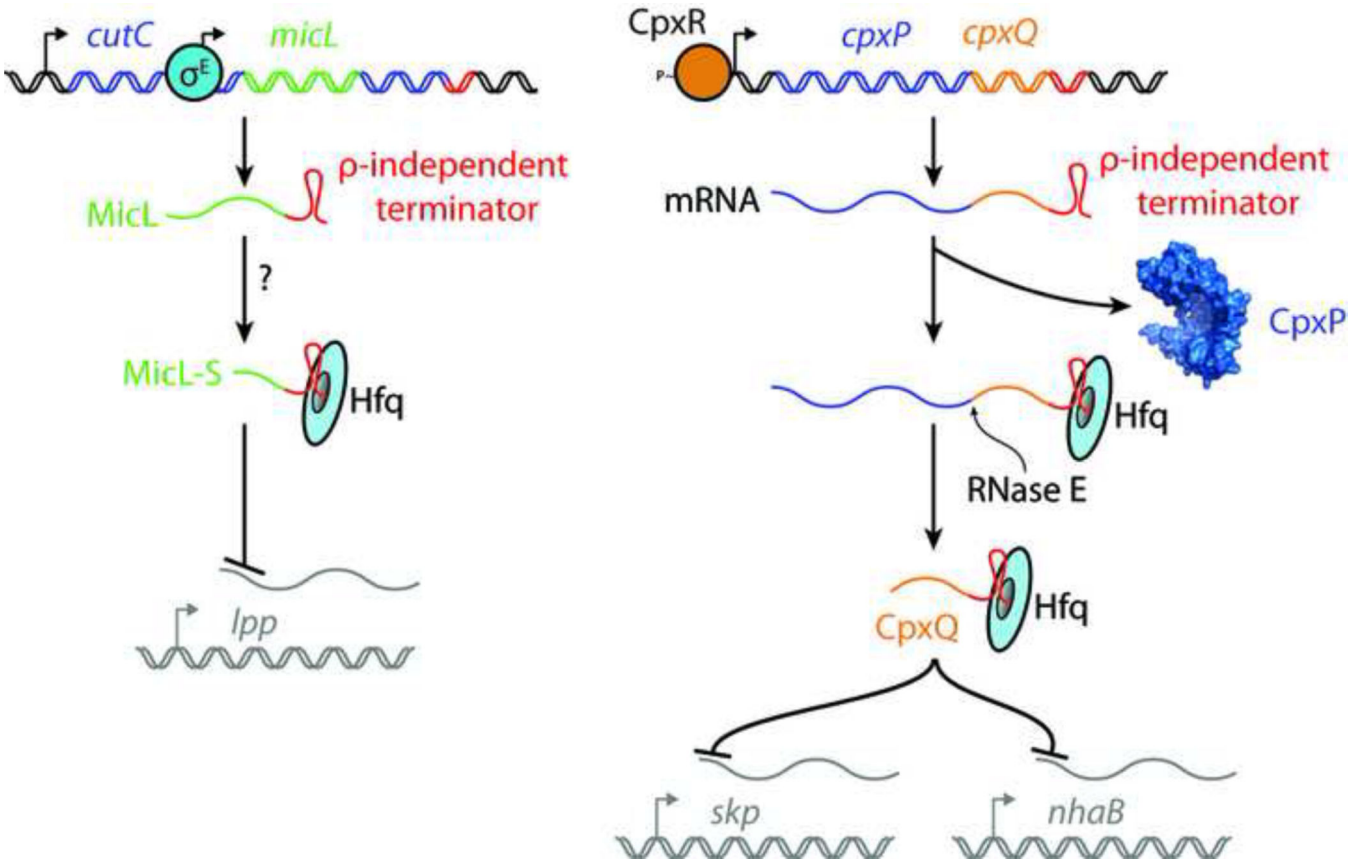

Figure 3. Biogenesis of 3' UTR sRNAs MicL and CpxQ

Left, the *micL* gene is entirely contained within 3' UTR of the gene annotated as *cutC*. *micL* transcription ends at the native *cutC* ρ-independent terminator. MicL is transcribed from a σ^E-dependent promoter within the *cutC* locus and then processed into a shorter product (MicL-S). Hfq binds the sRNA. Both MicL and MicL-S inhibit production of Lpp. Right, the CpxQ sRNA is a product of normal *cpxP* transcript degradation mediated by RNase E. CpxQ is stabilized by Hfq and is able to prevent expression from several mRNAs *in trans*, including *skp* and *nhaB*.

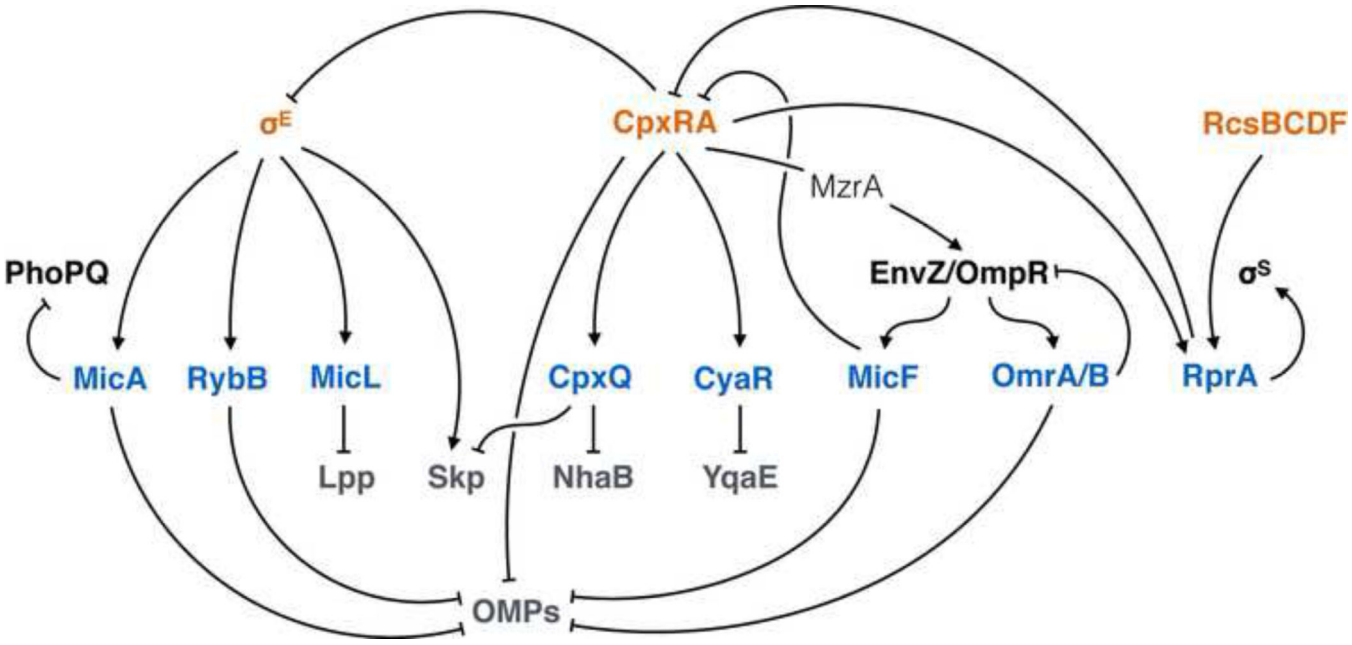

Figure 4. ESR sRNAs, their targets and the interconnected σ^E-Cpx network

The Cpx response can directly inhibit σ^E by CpxR binding and repression upstream of the *rpoE* gene. CpxR directly and potently represses expression of abundant OMPs, including OmpC and OmpF. CpxR also represses OMP synthesis indirectly using OmpR-regulated sRNAs; CpxR does this by inducing MzrA to stimulate the EnvZ/OmpR signal transduction pathway. CpxQ production inhibits synthesis of the σ^E effector Skp. MicA, RprA, and MicF each interconnect multiple signal transductions systems. Major ESRs are denoted in orange; other signal transduction systems are in black; sRNA products are in blue; gene targets are in grey.