MINIREVIEW

Just scratching the surface: an expanding view of the Cpx envelope stress response

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Abstract

To detect and effectively respond to damage to the cell envelope, Gram-negative bacteria possess multiple envelope stress responses. Among these, the CpxAR two-component system has been shown to sense the presence of misfolded periplasmic proteins and increase the production of envelope-localized protein folding and degrading factors in response. However, recent studies have revealed that additional parameters, such as adhesion and central metabolism, can also be sensed by the Cpx signalling system. The discovery that the Cpx regulon contains dozens to hundreds of genes indicates that the cellular functions of the Cpx response are also likely much broader than previously realized. These newly recognized functions include other aspects of envelope maintenance, communication with other regulatory pathways, and pathogenesis. A new model is emerging in which the Cpx response integrates diverse signals and promotes cell survival by protecting the envelope in multiple ways.

Introduction

To survive, all organisms must sense and respond to their environment. In bacteria, environmental signals are primarily sensed by two-component signal transduction (2CST) systems, consisting of a histidine kinase (HK), typically located in the inner membrane (IM), and a cytoplasmic response regulator (RR) (reviewed by Buelow & Raivio, 2010). When the HK detects a specific signal, it first autophosphorylates and then transfers the phosphate group to the RR, allowing the RR to act as a transcription factor to alter gene expression, in most cases. In the absence of an inducing signal, many HKs act as a phosphatase to maintain their cognate RRs in an inactive state. Studying 2CST systems is paramount to our understanding of bacterial adaptation, because these systems are the most widespread signalling pathways in nature (Wolanin et al., 2002). Although the Cpx 2CST system is among the most intensively studied, ongoing research continues to shed new light on its cellular role.

The Cpx system was first discovered when mutations in the chromosomal cpxA (conjugative pilus expression) locus were found to reduce expression of the F-plasmid conjugative pilus in Escherichia coli (McEwen & Silverman, 1980). Several years later, CpxA was identified by sequence analysis as a 2CST sensor protein (Nixon et al., 1986), with cpxR, the gene encoded immediately upstream of cpxA, demonstrated to encode its cognate RR (Dong et al., 1993; Raivio & Silhavy, 1997). In the 1990s, a series of studies established the view of Cpx as a novel envelope stress response. Mutations in cpxA were found to suppress the toxicity of secreted LamB-LacZ-PhoA fusion proteins, suggesting that activation of the Cpx system alleviates envelope protein misfolding (Cosma et al., 1995). In support of this idea, several envelope-localized protein folding and degrading factors were found to be Cpx-regulated, including the periplasmic protease and chaperone DegP (Danese et al., 1995), the disulphide bond oxidoreductase DsbA (Danese & Silhavy, 1997; Pogliano et al., 1997) and the peptidyl-prolyl isomerase PpiA (Pogliano et al., 1997). Other studies identified a number of signals capable of inducing the Cpx response. These include alkaline pH (Nakayama & Watanabe, 1995; Danese & Silhavy, 1998), alterations to the composition of the IM (Mileykovskaya & Dowhan, 1997; Danese et al., 1998) and the expression of uropathogenic E. coli (UPEC) Pap pilus subunits in the absence of their cognate chaperone (Jones et al., 1997). All of these inducing cues are believed to have the common feature of generating misfolded periplasmic and/or IM proteins. From these
results arose a model in which accumulation of misfolded envelope proteins activates CpxA, leading to the phosphorylation of CpxR and the upregulation of a suite of periplasmic chaperones and proteases that refold or degrade these misfolded proteins, thereby ameliorating the envelope stress.

Although these studies highlighted the importance of the Cpx response in E. coli’s ability to survive potentially lethal envelope protein misfolding, recent work has emphasized that this is only one facet of the Cpx system’s cellular role. Further examination of the signal sensing mechanism, regulon members and physiological functions of the Cpx pathway in E. coli and other bacteria has deepened our understanding of this important regulatory system.

**Signal sensing: multiple components integrate multiple signals**

There is a growing recognition that signal sensing by 2CST systems is not accomplished solely by the HK input domain; in fact, many 2CST systems integrate a number of inducing signals using various domains of both the HK and the RR, as well as auxiliary sensing proteins (reviewed by Buelow & Raivio, 2010). In the case of the Cpx system, at least four proteins in different cellular compartments participate in signal sensing: the outer membrane (OM) lipoprotein NlpE, the periplasmic protein CpxP, the IM HK CpxA and the cytoplasmic RR CpxR (Fig. 1).

NlpE (new lipoprotein E) was first identified as a multicopy suppressor of the toxicity of the envelope-localized LamB-LacZ-PhoA fusion protein (Snyder et al., 1995), with suppression being dependent on activation of the Cpx response (Danese et al., 1995). The physiological role of NlpE was not well understood until several years later, when Otto & Silhavy (2002) demonstrated that this protein is required for Cpx induction in response to adhesion to a hydrophobic surface. However, NlpE does not appear to be involved in sensing a variety of envelope stresses, such as alkaline pH or Pap subunit overexpression, because nlpE mutants retain their ability to activate the Cpx response in the presence of these cues (DiGiuseppe & Silhavy, 2003).

More recent studies have shed some light into the mechanism by which NlpE activates the Cpx response. Mutant NlpE constructs that are IM-localized, but not those localized to the periplasm, retain their Cpx-inducing capacity, showing that membrane association is critical for NlpE’s signalling function (Miyadai et al., 2004). X-ray crystallography of NlpE revealed that it forms a two-barrel structure, with the N-terminal barrel anchored in the OM (Hirano et al., 2007). Two possibilities for how NlpE, an OM lipoprotein, could potentially interact with CpxA in the IM have been proposed (Hirano et al., 2007). One possibility is that the N-terminal domain, which is inherently unstable, could unfold during surface adhesion, allowing the C-terminus of NlpE to directly contact the IM. Alternatively or in addition, when the periplasmic protein folding machinery is overloaded, NlpE might not fold properly, preventing recognition by the Lol transport machinery and therefore causing mislocalization of NlpE to the IM, thereby inducing the Cpx response.

There are hints that NlpE may be responsible for sensing other signals in addition to surface adhesion. nlpE was also identified in a screen for copper-sensitive E. coli mutants (Gupta et al., 1995). Intriguingly, the N-terminus of NlpE contains a CXXC motif that may be able to chelate copper ions (Hirano et al., 2007). NlpE also contains motifs with homology to the lipid-binding protein lipocalin, as well as an oligonucleotide/oligosaccharide-binding fold (Hirano et al., 2007). Therefore, NlpE could conceivably have the ability to detect a variety of envelope constituents, including lipids, lipopolysaccharide or peptidoglycan components. Furthermore, NlpE may not be the only auxiliary lipoprotein capable of inducing the Cpx response, as overexpression of the lipoproteins OsmB, Pal, NlpA and, in particular, YaF also increases expression of a degP-lacZ fusion (Miyadai et al., 2004). Whether induction of the Cpx response by these lipoproteins has a physiological role, and if so, what the cues sensed by these other lipoproteins are remain to be identified.

A second auxiliary regulator of CpxA is the periplasmic protein CpxP, which inhibits Cpx pathway activity when overexpressed (Raivio et al., 1999). Although direct evidence is still lacking, it is believed that this inhibition is mediated by protein–protein interaction between CpxP and the periplasmic domain of CpxA. In support of this hypothesis, inhibition by CpxP is lost when the periplasmic domain of CpxA is mutated (Raivio et al., 1999). Furthermore, the addition of CpxP to an in vitro reconstituted CpxA-CpxR system decreases the rate of CpxA autophosphorylation (Fleischer et al., 2007). The recent crystal structure of CpxP revealed a bowl-shaped dimer, with each protomer forming a long, bent and hooked hairpin (Zhou et al., 2011; Thede et al., 2011). The concave surface of the dimer is positively charged and has been proposed to interact with acidic residues present in the CpxA periplasmic domain (Zhou et al., 2011).

In the presence of envelope stress, the inhibitory function of CpxP is inactivated (DiGiuseppe & Silhavy, 2003). Under these conditions, CpxP may be titrated away from CpxA through binding to misfolded proteins like pilins (Isaac et al., 2005). CpxP also becomes a substrate for the DegP protease under Cpx-inducing conditions (Buelow & Raivio, 2005; Isaac et al., 2005). Proteolysis of CpxP is an
important component of the Cpx response, as the Cpx pathway cannot be fully activated in a \textit{degP} mutant (Buelow & Raivio, 2005). Interestingly, there is no change in the dimerization state of CpxP and only minor alterations in its conformation at alkaline pH, an inducing condition, suggesting that Cpx-inducing conditions may affect CpxP’s ability to interact with partners like CpxA without causing large rearrangements in its structure (Thede et al., 2011). The role of CpxP in signal sensing is poorly understood. CpxP is not responsible for detecting known Cpx-specific envelope stresses, because \textit{cpxP} mutants retain their ability to sense NlpE overexpression, alkaline pH, PapE and PapG overexpression, and other stresses (Raivio et al., 1999; DiGiuseppe & Silhavy, 2003). CpxP could therefore be responsible for fine-tuning Cpx activation, by preventing inappropriate induction of CpxA and allowing rapid shut-off of the Cpx response once envelope stress is relieved (Raivio et al., 1999). Alternatively, CpxP could be capable of sensing a signal that has not yet been identified. It is interesting to note that CpxP has structural homology to periplasmic metal-binding proteins such as CnrX and ZraP, and that zinc ions were found in the CpxP crystal structure (Thede et al., 2011). The role of CpxP in metal ion sensing therefore merits further research. The crystal structure of CpxP is also similar to the recently solved structure of Spy, a periplasmic protein that is positively regulated by the Cpx response (Kwon et al., 2010; Quan et al., 2011). Despite the structural similarity, Spy does not share CpxP’s ability to inhibit Cpx pathway activation (Raivio et al., 2000; Buelow & Raivio, 2005); rather, Spy functions as an ATP-independent periplasmic chaperone (Quan et al., 2011). As might be expected from the structural similarity, CpxP also displays a modest chaperone activity, in addition to its signalling role (Zhou et al., 2011; Quan et al., 2011). The HK CpxA represents a major signal integration point. The periplasmic domain of CpxA is required for phosphatase on the RR CpxR, keeping it dephosphorylated and therefore inert. CpxP inhibits activation of CpxA, possibly through a direct interaction. Inducing cues enter the Cpx pathway at several points (indicated by blue arrows): surface adhesion is sensed by the OM lipoprotein NlpE, misfolded envelope proteins may be sensed directly by CpxA, and growth/metallic cues are sensed by CpxR. Under inducing conditions (right side of diagram), the inhibitory molecule CpxP is degraded by DegP, CpxA acts as an autokinase and a CpxR kinase, and phosphorylated CpxR binds to DNA to regulate transcription. Target genes upregulated by CpxP-P (indicated by green text) include those encoding periplasmic protein folding and degrading factors, peptidoglycan metabolic enzymes, and some IM proteins and regulators. Targets downregulated by CpxP-P (indicated by red text) include envelope-localized protein complexes and other IM proteins and regulators.

\begin{figure}
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\caption{Overview of the Cpx pathway in \textit{Escherichia coli}. Under non-inducing conditions (left side of diagram), the CpxA HK acts as a phosphatase on the RR CpxR, keeping it dephosphorylated and therefore inert. CpxP inhibits activation of CpxA, possibly through a direct interaction. Inducing cues enter the Cpx pathway at several points (indicated by blue arrows): surface adhesion is sensed by the OM lipoprotein NlpE, misfolded envelope proteins may be sensed directly by CpxA, and growth/metallic cues are sensed by CpxR. Under inducing conditions (right side of diagram), the inhibitory molecule CpxP is degraded by DegP, CpxA acts as an autokinase and a CpxR kinase, and phosphorylated CpxR binds to DNA to regulate transcription. Target genes upregulated by CpxP-P (indicated by green text) include those encoding periplasmic protein folding and degrading factors, peptidoglycan metabolic enzymes, and some IM proteins and regulators. Targets downregulated by CpxP-P (indicated by red text) include envelope-localized protein complexes and other IM proteins and regulators.}
\end{figure}
both induction by NlpE (Raiovio & Silhavy, 1997) and inhibition by CpxP (Raiovio et al., 1999). Mutations in the periplasmic domain of CpxA also prevent detection of envelope stresses such as alkaline pH, PapE and PapG overexpression, and envelope perturbation by EDTA (DiGiuseppe & Silhavy, 2003), all of which are sensed independently of CpxP and NlpE. It is therefore possible that CpxA can directly sense some feature of misfolded envelope proteins, the nature of which has not been identified. There is some specificity to the signal, as the overexpression of only certain Pap pilins activates the Cpx response, and Cpx activation does not correlate directly with pilin aggregation (Lee et al., 2004). In support of CpxA’s ability to directly sense misfolded proteins, the MalE219 mutant protein is capable of increasing the rate of phosphotransfer from CpxA to CpxR in an in vitro assay (Keller & Hunke, 2009). However, in most cases, it is formally possible that CpxA-dependent signal sensation could involve another, currently unknown auxiliary protein(s).

The function of conserved residues in the CpxA periplasmic domain has recently been analysed using alanine substitution mutations (Malpica and Raiovio, in preparation). Strikingly, virtually all of the substitutions with a mutant phenotype led to increased Cpx pathway activity, even under noninducing conditions. These results suggest that the Cpx response is activated by default, with mutations leading to a loss of phosphatase function and/or elevated kinase activity and therefore increased Cpx pathway activity. It is possible that misfolded proteins could interact with some of the inhibitory residues in the CpxA periplasmic domain to allow CpxA to adopt an activated conformation. Alternatively, these residues could interact with CpxP or other, currently unidentified inhibitory proteins. The removal of these inhibitory interactions in the presence of activation signals could then be responsible for induction of the pathway.

Finally, cytoplasmic or growth signals can be integrated into the Cpx pathway downstream of CpxA, through CpxR. The expression of cpxRA is activated at the onset of stationary phase (De Wulf et al., 1999), and in E. coli strain MC4100, this growth-related activation is CpxR-dependent but CpxA-independent (DiGiuseppe & Silhavy, 2003). CpxR can also be activated independently of CpxA when cells are grown in the presence of excess carbon, such as glucose or pyruvate (Wolfe et al., 2008). This is believed to occur via the Pta-AckA pathway, which generates acetyl phosphate from acetyl-CoA (Wolfe et al., 2008). Acetyl phosphate itself can phosphorylate CpxR in vitro (Pogliano et al., 1997; Raiovio & Silhavy, 1997) and under particular growth conditions in vivo (Wolfe et al., 2008). Additionally, other indirect products of the Pta-AckA pathway can influence the CpxR-dependent transcription of cpxP (Wolfe et al., 2008), with acetylation of residue K298 in the α subunit of RNA polymerase playing a role in this activation (Lima et al., 2011). Although the mechanism is not fully understood, it is clear that CpxR is capable of sensing signals related to growth and central metabolism without the involvement of CpxA.

Cpx regulon: more than envelope protein quality control

The list of target genes regulated by CpxR has also undergone a recent expansion. Although genes associated with envelope protein folding and degradation constitute the most strongly regulated members of the Cpx regulon (Price & Raiovio, 2009), a recent microarray analysis shows that the Cpx regulon may contain several hundred genes (Price and Raiovio, in preparation), clearly indicating a broader cellular role. The newly identified Cpx regulon members fall into several functional categories, including envelope protein complexes, IM proteins, peptidoglycan metabolic enzymes and other cellular regulators (Fig. 1).

Although the first identified Cpx regulon members were all positively regulated by CpxR, microarray analysis reveals that the Cpx regulon contains approximately equal numbers of upregulated and downregulated genes (Bury-Mone et al., 2009; Price and Raiovio, in preparation). One category of downregulated genes is those involved with the biogenesis of envelope-localized protein complexes such as pili and flagella. The mechanisms by which this downregulation is achieved, however, are diverse. Mutations in cpxA that constitutively activate the Cpx response render cells incapable of elaborating conjugal F-pili (McEwen & Silverman, 1980; Silverman et al., 1993). This downregulation is mediated at the level of protein stability, through degradation of the transcriptional activator TraJ by the Cpx-regulated protease HslVU (Gubbins et al., 2002; Lau-Wong et al., 2008). On the other hand, CpxR downregulates expression of the curli fimbriae both directly and indirectly. CpxR directly represses expression of the csgBA operon, encoding the major curli subunit CsgA. Further repression of the csgBA operon is achieved indirectly through the CpxR-mediated inhibition of expression of the csgDEFG operon, which encodes the major transcriptional activator of curli expression, CsgD (Dorel et al., 1999; Prigent-Combaret et al., 2001; Jubelin et al., 2005; Ogasawara et al., 2010). Flagellar motility of E. coli K-12 is also decreased by the Cpx response (De Wulf et al., 1999). Regulation of motility appears to occur at several levels. CpxR directly represses expression of the motABcheAW, trs and aer genes, encoding components of the flagellar motor and chemotaxis and aerotaxis proteins (De Wulf et al., 1999, 2002). Microarray results also suggest that expression of the flagellar master regulator FlhC

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is downregulated in response to overexpression of NlpE (Price and Raivio, in preparation). Although the downregulation of various pilus, flagella and additional virulence-related envelope structures (discussed later) by the Cpx response is clear, the rationale for regulation of these genes is uncertain. Downregulation of nonessential protein complexes may relieve the burden on the envelope protein folding machinery when misfolded proteins are already abundant (MacRitchie et al., 2008a). Alternatively or in addition, the repression of these energy-intensive structures may help to conserve finite cellular resources during times of stress (De Wulf et al., 1999).

There is also a growing appreciation of the connection between the Cpx response and IM proteins. Many of the originally identified Cpx-inducing cues, such as Pap pilus subunit and NlpE overexpression, result in the aggregation of misfolded proteins at the periplasmic face of the IM (Jones et al., 1997; Miyadai et al., 2004). Additionally, Shimohata et al. (2002) showed that the Cpx response is activated by mutation of the IM protease-encoding gene ftsH, and that in response, CpxR upregulates expression of htpX, encoding another IM protease. These results suggest that the Cpx response can sense abnormalities of integral IM proteins caused by the lack of FtsH and respond by regulating IM proteolysis. In support of a role for the Cpx response in regulating IM proteolysis, another recently characterized Cpx-regulated IM protein is YccA, which aids cell survival when protein translocation is stalled by preventing FtsH-mediated proteolysis of the Sec complex (van Stelten et al., 2009). Microarray analysis of the genes affected by overexpression of NlpE revealed an enrichment for IM proteins (Price and Raivio, in preparation). Included among these IM proteins are numerous transporters for a variety of substrates, such as fatty acids, amino acids and ions, most of which were downregulated (Price and Raivio, in preparation). Together, these observations may suggest that the function of the Cpx response is tightly linked to the status of the IM and/or its protein content. Because many of the Cpx-regulated IM proteins identified by microarrays have currently unknown functions (Bury-Moné et al., 2009; Price and Raivio, in preparation), the cellular impact of Cpx regulation of IM proteins is yet to be fully understood.

An additional envelope constituent that appears to be affected by the activation of the Cpx response is the peptidoglycan of the cell wall. Weatherspoon-Griffin et al. (2011) have recently shown that CpxR directly activates expression of amiA and amiC, genes encoding two N-acetylmuramoyl-l-alanine amidases that cleave peptide crossbridges from N-acetylMuramic acid residues to allow daughter cell separation during cell division. Interestingly, amiA and amiC mutants are characterized by increased OM permeability (Ize et al., 2003; Weatherspoon-Griffin et al., 2011), suggesting that CpxR regulation of these genes may function to improve the integrity of the cell envelope. A similar role may be played by the Cpx-regulated protein YcfS, which is an L,D-transpeptidase that links peptidoglycan to the OM lipoprotein Lpp (Yamamoto & Ishihama, 2006; Magnet et al., 2007; Price & Raivio, 2009). A number of other proteins with known or predicted roles in peptidoglycan metabolism are upregulated by the overexpression of NlpE (Price and Raivio, in preparation), which may indicate peptidoglycan remodelling during the Cpx response.

Another factor likely contributing to the relatively large size of the Cpx regulon is that several other cellular regulators appear to be under the control of CpxR. Surprisingly, CpxR negatively and directly regulates the expression of the rpoErseABC operon, which encodes the alternative sigma factor σE, the mediator of an additional envelope stress response that detects OM protein misfolding (De Wulf et al., 2002; Price & Raivio, 2009). The cellular benefit of downregulating another envelope stress response is unknown, but could suggest that some σE regulon members perform functions that are detrimental under Cpx-inducing conditions (Price & Raivio, 2009). CpxR also interfaces with the EnvZ/OmpR 2CST system, in this case via positive regulation of the small, IM-localized protein MzrA (Gerken et al., 2009). MzrA and EnvZ physically interact via their periplasmic domains (Gerken & Misra, 2010). This interaction increases the expression of genes in the OmpR regulon in an EnvZ- and OmpR-dependent manner, presumably by either increasing EnvZ phosphorylation of OmpR or decreasing EnvZ phosphatase activity or both (Gerken et al., 2009). Positive regulation of MzrA therefore allows CpxR to communicate with EnvZ-OmpR without cross-phosphorylation by non-cognate HK-RR pairs, which has been shown to be kinetically unfavourable (Siryporn & Goulian, 2008; Groban et al., 2009). Another regulatory protein that is positively regulated by CpxR is YdeH, a diguanylate cyclase capable of synthesizing the signalling molecule cyclic di-GMP (Yamamoto & Ishihama, 2006; Jonas et al., 2008; Price & Raivio, 2009). YdeH both inhibits motility and promotes biofilm formation (Jonas et al., 2008; Boehm et al., 2009). These connections with other cellular regulatory networks therefore allow the Cpx response to affect a variety of complex bacterial behaviours.

**Physiological function: a role for the Cpx response in pathogenesis**

Because many structures critical for bacterial virulence reside in the envelope, it is unsurprising that the Cpx response affects the ability of numerous Gram-negative
pathogens to infect their hosts. Early results suggested that the Cpx response might enhance virulence by increasing the expression of periplasmic protein folding factors such as DsbA that are required for the assembly of cell-surface structures like pili (Peek & Taylor, 1992; Jacob-Dubuisson et al., 1994; Zhang & Donnenberg, 1996). Other Cpx regulon members appear to contribute to cell-surface structure expression as well; for example, both DegP and CpxP are required for efficient elaboration of the enteropathogenic E. coli (EPEC) type IV bundle-forming pilus (BFP) (Vogt et al., 2010; Humphries et al., 2010). In accordance with these findings, inactivation of the Cpx response adversely affects assembly of some pili. When the UPEC Pap pili genes are expressed in E. coli K-12, mutation of cpxR results in the production of shorter pili and a higher proportion of cells that do not express any pili because of phase variation (Hung et al., 2001). Likewise, expression of the BFP pilin bundle and adherence to cultured human cells is reduced in an EPEC cpxR mutant (Nevesinjac & Raivio, 2005).

Studies in several other organisms revealed that the Cpx response has important virulence-related functions beyond its role in pilus elaboration (Table 1). In Shigella spp., the Cpx response controls the expression of two key virulence regulators, VirF and InvE (Nakayama & Watanabe, 1995; Nakayama & Watanabe, 1998; Mitobe et al., 2005). The expression of virF, which encodes a positive regulator of type III secretion genes, is enhanced by the direct binding of CpxR to its promoter (Nakayama & Watanabe, 1998). In an interesting example of post-transcriptional regulation by the Cpx response, the protein levels of InvE, but not its mRNA abundance, are decreased in a cpxA mutant of Shigella sonnei, in which the Cpx response is presumably constitutively activated (Mitobe et al., 2005). In Legionella pneumophila, CpxR has been shown to positively regulate the transcription of numerous components of the Icm/Dot type IV secretion system and its substrates, including the chaperone IcmR (Gal-Mor & Segal, 2003); the structural subunits IcmV, IcmW, DotA and LvgA (Vincent et al., 2006; Altman & Segal, 2008); and a host of newly identified Icm/Dot translocated substrates (Altman & Segal, 2008). Curiously, mutations in either cpxR or cpxA have no effect upon L. pneumophila intracellular growth within macrophages or amoebae (Gal-Mor & Segal, 2003). The benefit of Cpx regulation of type IV secretion in L. pneumophila therefore remains to be determined.

In contrast to these results, recent studies have suggested that in many pathogens, activation of the Cpx response is detrimental to virulence (Table 1). In several

### Table 1. Virulence phenotypes associated with Cpx response mutations

<table>
<thead>
<tr>
<th>Organism</th>
<th>Virulence phenotype</th>
<th>Reference</th>
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<tr>
<td><strong>Cpx response promotes virulence</strong></td>
<td></td>
<td></td>
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<tr>
<td>Enteropathogenic</td>
<td></td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>cpxR null mutant has decreased elaboration of bundle-forming pilus and decreased adherence to cultured cells</td>
<td>Nevesinjac &amp; Raivio (2005)</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>cpxR null mutation abolishes expression of T3S transcriptional regulator VirF; cpxA deletion post-transcriptionally reduces expression of InvE regulator</td>
<td>Nakayama &amp; Watanabe, 1998; Mitobe et al. (2005)</td>
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<tr>
<td><em>Legionella pneumophila</em></td>
<td>CpxR directly activates expression of several Icm/Dot type IV secretion system components, as well as some secreted substrates</td>
<td>Gal-Mor &amp; Segal (2003); Vincent et al. (2006); Altman &amp; Segal (2008)</td>
</tr>
<tr>
<td><em>Xenorhabdus nematiphila</em></td>
<td>cpxR mutant has reduced virulence in the insect host Manduca sexta, likely related to its decreased growth rate in <em>insecta</em>, increased stimulation of antimicrobial peptide production and reduced expression of the pathogenesis regulator LrhA</td>
<td>Herbert et al. (2007); Herbert Tran &amp; Goodrich-Blair (2009)</td>
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<tr>
<td><strong>Cpx response inhibits virulence</strong></td>
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<tr>
<td>Enteropathogenic</td>
<td>Activation of Cpx response inhibits bundle-forming pilus expression, type III secretion and motility</td>
<td>MacRitchie et al. (2008b); Vogt et al. (2010); MacRitchie et al. (unpublished)</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> serovar</td>
<td>cpxA* constitutively active mutation inhibits adherence to cultured cells and reduces virulence in mice</td>
<td>Humphreys et al. (2004)</td>
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<tr>
<td><em>Typhimurium</em></td>
<td>cpxA mutation inhibits type III secretion and adherence to host cells, via downregulation of the adhesin invasin</td>
<td>Carlsson et al. (2007a, b)</td>
</tr>
<tr>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>CpxR inhibits expression of several Icm/Dot type IV-secreted substrates</td>
<td>Altman &amp; Segal (2008)</td>
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<tr>
<td><em>Legionella pneumophila</em></td>
<td>Cpx inhibits expression of several virulence determinants, including LspB-LspA2 and DsrA, <em>ΔcpxA</em> pathway-activating mutation renders <em>H. ducreyi</em> avirulent in human volunteers</td>
<td>Labandeira-Rey et al. (2009); Spinola et al. (2010); Labandeira-Rey et al. (2010)</td>
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*Organism in which Cpx response has both positive and negative effects on virulence.
organisms, mutations in cpxA, which in many cases result in an accumulation of phosphorylated CpxR (Wolfe et al., 2008; Malpica and Raivio, in preparation), have been found to decrease expression of adhesins and adherence to host cells. For example, expression of the EPEC BFP, the UPEC Pap pilus and invasin, a nonfimbrial adhesin produced by Yersinia pseudotuberculosis, is decreased in cpxA mutant strains (Hernday et al., 2004; Carlsson et al., 2007b; Vogt et al., 2010). In addition, a Salmonella enterica serovar Typhimurium cpxA mutant has defects in host cell adherence, although the specific adhesin affected in this strain was not determined (Humphreys et al., 2004). The Cpx response therefore appears to have a conserved role in the repression of adhesive structures. Expression of several virulence-associated protein secretion systems is also reduced by mutations in cpxA, including the EPEC and Yersinia enterocolitica type III secretion systems and the Haemophilus ducreyi LspB-LspA2 two-partner secretion system (Carlsson et al., 2007a; MacRitchie et al., 2008b; Labandeira-Rey et al., 2009). Accordingly, the S. Typhimurium and H. ducreyi cpxA mutants were also found to be less virulent in infection models (Humphreys et al., 2004; Spinola et al., 2010). As suggested earlier, this repression of adhesive structures and secretion systems by the Cpx response may be a pre-emptive mechanism to prevent further envelope protein misfolding. Alternatively, it is possible that the Cpx response plays a critical role in the life cycle of these pathogens by repressing expression of adhesive cell-surface structures to allow for detachment from the site of infection and transmission to new hosts or infection sites. These possibilities remain to be investigated.

One model system that shows promise in revealing the role of the Cpx response in bacterium–host interactions involves the organism Xenorhabdus nematophila. X. nematophila associates mutualistically with the entomopathogenic nematode Steinernema carpocapsae; the bacterium and the nematode cooperate in colonizing the insect host (Chaston & Goodrich-Blair, 2010). Interestingly, inactivation of the Cpx response reduces the ability of X. nematophila to both colonize its nematode host and successfully infect an insect host (Herbert et al., 2007). Subsequent studies determined that the nematode colonization defect of the cpxR mutant likely results from diminished expression of the envelope-localized colonization factors NiIA, NiIB and NiIC (Herbert Tran et al., 2009), while the virulence defect could be the result of insufficient expression of the virulence-related transcriptional regulator LrhA (Herbert Tran & Goodrich-Blair, 2009). It therefore appears that the Cpx response has important functions in multiple stages of the X. nematophila life cycle. Further studies in this pathogen and others will undoubtedly improve our understanding of the role of the Cpx response in bacterium–host interactions.

**Conclusion**

It is now clear that the Cpx envelope stress response represents more than simply a means to detect and repair misfolded periplasmic proteins. A variety of signals can enter the Cpx signalling pathway at multiple points, with NlpE sensing adhesion, CpxA possibly sensing misfolded envelope proteins, and CpxR sensing growth and metabolism. A variety of target genes are regulated by phosphorylated CpxR, including those encoding envelope protein complexes, IM proteins, peptidoglycan metabolic enzymes and other regulators. Finally, the Cpx response regulates virulence processes in numerous pathogens (Table 1). Most of these inducing cues and regulatory targets still pertain to the cell envelope, validating the original characterization of CpxAR as an envelope stress response; however, the Cpx response also promotes envelope function in diverse ways not previously recognized (summarized in Fig. 1).

In spite of these advances, many questions remain. What is the molecular nature of the signal sensed by CpxA, and are there additional auxiliary signal sensing proteins that feed into CpxA? What cellular functions are performed by the many poorly characterized members of the Cpx regulon? Do any of these proteins represent connectors to other regulatory pathways, similar to MzrA? Finally, for pathogens capable of living outside of a host, is the Cpx response activated during the infection process, or is it primarily important for survival in external environments? Recent technical advances promise that these questions will soon be addressed, likely prompting a further expansion in our understanding of the Cpx envelope stress response.

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