Regulation of *Escherichia coli* cell envelope proteins involved in protein folding and degradation by the Cpx two-component system

Joe Pogliano,1,4 A. Simon Lynch,1,3 Dominique Belin,2 E.C.C. Lin,1 and Jon Beckwith1

1Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115 USA; 2Department de Pathologie, Centre Medical Universitaire, 1211 Geneva 4, Switzerland

We show that the two-component signal transduction system of *Escherichia coli*, CpxA–CpxR, controls the expression of genes encoding cell envelope proteins involved in protein folding and degradation. These findings are based on three lines of evidence. First, activation of the Cpx pathway induces 5- to 10-fold the synthesis of DsbA, required for disulfide bond formation, and DegP, a major periplasmic protease. Second, using electrophoretic mobility shift and DNase I protection assays, we have shown that phosphorylated CpxR binds to elements upstream of the transcription start sites of *dsbA*, *degP*, and *ppiA* (*rotA*), the latter coding for a peptidyl-prolyl *cis/trans* isomerase. Third, we have demonstrated increased in vivo transcription of all three genes, *dsbA*, *degP*, and *ppiA*, when the Cpx pathway is activated. We have identified a putative CpxR consensus binding site that is found upstream of a number of other *E. coli* genes. These findings suggest a potentially extensive Cpx regulon including genes transcribed by σ70 and σ5, which encode factors involved in protein folding as well as other cellular functions.

[Key Words: DsbA; Cpx; DegP; protein folding; transcription]

Received January 28, 1997; revised version accepted March 24, 1997.

A number of proteins have been identified in bacteria that catalyze different steps in the folding of cell envelope proteins. Periplasmic proteins catalyze disulfide bond formation, the isomerization of peptidyl-proline bonds, and protein degradation, and may be important for the assembly of outer membrane proteins [Wulfing and Pluckthun 1994]. Under certain conditions, cell envelope proteins may be more exposed to environmental stresses than cytoplasmic proteins, making the need for protein maintenance and repair systems specific for the cell envelope important for survival [Visick and Clarke 1995]. However, very little is known about how bacteria regulate the expression of periplasmic protein folding catalysts.

In *Escherichia coli*, the best understood periplasmic protein folding pathway is the Dsb system that catalyzes disulfide bond formation. The DsbA and DsbB proteins are required for the formation of disulfide bonds in cell envelope proteins [Bardwell et al. 1991, 1993; Kamitani et al. 1992; Jander et al. 1994; Guilhot et al. 1995]. The DsbC protein is apparently also involved in cell envelope protein folding as it mediates the shuffling of disulfide bonds when incorrect disulfide bonds have formed [Rietsch et al. 1996].

In addition to proteins containing disulfide bonds, those containing proline residues may also require the activity of folding catalysts for efficient folding. Peptidyl-proline residues can occur in either the *cis* or *trans* conformations, yet usually only one conformation is suitable to yield a correctly folded protein [Wulfing and Pluckthun 1994]. *E. coli* contains at least three periplasmic proteins catalyzing isomerization, peptidyl-prolyl *cis/trans* isomerases [PPlases] or rotamases, each belonging to a different family: PpiA (cyclophilin family) [Liu and Walsh 1990; Hayano et al. 1991], FkpA (FKBP family) [Roof et al. 1994; Wulfing et al. 1994; Horne and Young 1995], and SurA [PpiC family] [Rahfeld et al. 1994; Rudd et al. 1995; Lazar and Kolter 1996; Missiakas et al. 1996; Rouvière and Gross 1996]. Although all of the genes encoding these enzymes are nonessential, PpiA is a potent PPlase in vitro [Liu and Walsh 1990; Hayano et al. 1991], and SurA stimulates the rate of folding of the outer membrane protein LamB in vivo [Lazar and Kolter 1996; Missiakas et al. 1996; Rouvière and Gross 1996].

If any of these periplasmic protein-folding catalysts is regulated, a mechanism [or mechanisms] must exist for
sensing either cell envelope protein-folding defects or the conditions that lead to folding defects and for transmitting these signals across the inner membrane. A stress response pathway involved in the maturation of cell envelope proteins has been identified recently in E. coli. This pathway requires σE, a member of the ECF (extra cytoplasmic function) family of transcription factors, as well as at least two Rse (regulator of sigma E) proteins. σE Activity is induced by heat shock, by the overproduction of outer membrane proteins, and by surA mutations [Mecsas et al. 1993; Raina et al. 1995; Rouviere et al. 1995]. Recent evidence suggests that the transmembrane protein RseA is an anti-σ factor whose activity is regulated by the periplasmic protein RseB [De Las Peñas et al. 1997].

The σE regulon includes at least 10 genes, based on the analysis of two-dimensional gels after overproduction of σE, but only three of these have been identified: degP, rpoH (σ32), and rpoE [Raina et al. 1995; Rouviere et al. 1995]. The degP gene encodes a protease protease induced by heat shock [Lipinska et al. 1988; Strauch and Beckwith 1988]. Although the cellular substrates of DegP are not known, certain unstable foreign proteins are stabilized by degP mutations [Strauch et al. 1989]. The rpoH gene encodes the heat shock sigma factor, σ32, which directs transcription of the genes encoding the classical heat shock response, such as the chaperones DnaK/J and GroES/EL.

In bacteria, sensing and responding to external stimuli is usually mediated by two-component signal transduction systems [for review, see Parkinson and Kofoed 1992], and one might therefore expect such a system to play a role in both detecting cell envelope defects and coordinating the appropriate response. The Cpx system, composed of the CpxA sensor kinase and the CpxR response regulator [Dong et al. 1993], represents a possible candidate for such a system. The cpx locus was first identified by dominant mutations in the cpxA gene that adversely affect a number of cell envelope processes, such as the ability to transport small molecules into the cell via proton-driven cotransporters, F plasmid conjugation, and the production of outer membrane proteins [for summary, see Rainwater and Silverman 1990]. More recently, these so-called cpxA* mutations were found to confer resistance to the toxic effects associated with the expression and export of a hybrid protein [Cosma et al. 1995; Snyder et al. 1995] and lead to constitutively high expression from the degP promoter [Danese et al. 1995]. Furthermore, the wild-type Cpx proteins stimulate transcription of the degP gene in response to overexpression of NlpE [Danese et al. 1995], a lipoprotein of unknown function [Gupta et al. 1995]. In addition to CpxA and CpxR, σE is required for induction of degP transcription [Danese et al. 1995].

Because σE appears to function as an extracytoplasmic periplasmic stress response pathway and Cpx and σE together control expression of degP transcription, it has been suggested that the Cpx proteins are also part of a stress response pathway involved in monitoring periplasmic protein folding [Cosma et al. 1995]. However, the relationship between Cpx and σE is unclear. For example, Cpx could regulate degP expression indirectly by altering the activity or expression of the proposed regulators of σE, namely RseAB. Alternatively, Cpx could directly activate σE-transcribed genes by binding to sequences adjacent to σE-dependent promoters. Finally, Cpx could play a more general role in cell physiology by controlling the transcription of genes transcribed by other sigma factors, such as σ70, in addition to σE. Here, we identify new targets of the Cpx signal transduction pathway and provide evidence that CpxA and CpxR serve to directly regulate the expression of genes encoding periplasmic protein-folding catalysts. We also demonstrate that phosphorylated CpxR binds to specific sequences upstream of both σE and σ70 promoters, allowing the identification of a putative DNA-binding consensus sequence and suggesting that CpxA and CpxR comprise an extracellular stress response that possibly controls the expression of a large number of genes in E. coli.

Results

Temperature-regulated expression of DegP in cpx*, Δcpx, and cpxA* mutants

Dominant, gain-of-function cpxA* mutations that result in pleiotropic phenotypes have been suggested to lock the CpxA protein into a conformation that results in constitutive signaling in the absence of the normal stimulus or ligand. Consistent with this idea, cpxA* mutations lead to constitutive, high-level expression of the heat-inducible degP gene [Danese et al. 1995]. Expression of DegP is greatly increased in mutants containing cpxA* mutations [Fig. 1]. Because both temperature and Cpx appear to regulate the expression of DegP, we examined the Cpx dependence of the effect of temperature on the expression of DegP [Fig. 1]. The rates of synthesis of the DegP protein in a variety of cpxA mutants at 30°C, 37°C, or after a 10-min shift from 30°C to 42°C were measured in pulse-chase immunoprecipitation experiments, and the quantitated results are shown in Figure 1. In wild-type cultures, synthesis of DegP is slightly elevated (~50%) when grown at 37°C compared with 30°C, and DegP is induced eightfold after 10 min of heat shock. In cpxA* mutants, rates of DegP synthesis are significantly elevated at 37°C compared with wild type and increase even further after a shift to 42°C. The degree of regulation of DegP synthesis by temperature in a cpxA null mutant is identical to that seen with regulation in cpx* strains. Hence, the pathway leading to activation of σE by temperature appears to be independent of Cpx.

DsbA is overexpressed in mutants containing cpxA* mutations

On the basis of experiments showing that cpxA* mutants constitutively express the DegP protease, we reasoned that CpxA may be a sensor of cell envelope defects and that other genes involved in maturation of cell envelope proteins may therefore be regulated by Cpx. One prediction of this model is that periplasmic protein-fold-
DegP expression in cpx+, Δcpx, and cpxA* mutants. Pulse-chase experiments were performed at 30°C (solid bars), 37°C (hatched bars), or after a 10-min shift from 30°C to 42°C (shaded bars) as described in Materials and Methods. Strains are JP406 (cpxRA+), ECL1212 (ΔcpxRA), JP408 (cpxA9), JP467 (cpxA2.1), and JP468 (cpxA9.4). The data shown is from a single, representative experiment. Results using another cpx+ wild-type strain (JP466) were similar.

DsbA is regulated by the wild-type Cpx signal transduction pathway

In addition to being regulated by cpxA* mutations, the degP gene is regulated by the wild-type Cpx proteins in response to overproduction of an exported lipoprotein, NlpE [Danese et al. 1995]. We examined the ability of NlpE overexpression to induce synthesis of the DsbA and DegP proteins using pulse-chase immunoprecipitation experiments. A plasmid, pND18, expressing NlpE from the arabinose promoter, induces the DegP protein approximately four-fold (Fig. 2b). Furthermore, the induction of DegP protein by NlpE was found to be dependent on the Cpx proteins. These results are in accord with those of previous studies based on strains containing a λ phage expressing a degP-lacZ transcriptional fusion, suggesting that both methods accurately reflect changes in transcription from the degP promoter in vivo. The rates of synthesis of DsbA were increased approximately four-fold after 1.25 hr of production of NlpE (Fig. 2a). Overproduction of NlpE did not result in DsbA induction if the cpx operon was deleted. These results suggest that expression of DsbA is regulated by the Cpx pathway.

Cpx regulates transcription of the dsbA gene

The dsbA gene is transcribed from two promoters: a proximal promoter (P1) immediately upstream of the dsbA open reading frame and a distal promoter (P2) up-

Figure 2. DegP and DsbA expression in cpxA* mutants or in cpx+ and Δcpx null strains when NlpE is expressed from a high-copy-number plasmid. The genotype of each strain is indicated as + for cpx+, Δ for Δcpx, and * for cpxA*. Strains 1–4 contain the plasmid pND18 or pBAD22 and were grown with [solid bars] or without [hatched bars] arabinose. Strains 5, 6, and 7 do not contain plasmids and were not grown in the presence of arabinose. Cultures grown at 37°C in minimal maltose medium were either induced [solid bars] or not induced [hatched bars] with 0.2% arabinose for 1.25 hr and then pulse-labeled as described in Materials and Methods. Strain 1 [JP472]; strain 2 [JP474]; strain 3 [JP558]; strain 4 [JP560]; strain 5 [JP406]; strain 6 [JP408]; strain 7 [JP467]. The results shown are from a typical experiment. Similar results were obtained using a different time of induction of NlpE with arabinose (1.5 hr) or using a different strain background in which cells cannot catalyze arabinose [JP313].
stream of an open reading frame (yihE) that precedes the dsbA gene (Belin and Boquet 1994). The DsbA protein might be regulated by Cpx directly by stimulating transcription of one or both of the dsbA promoters or indirectly by another mechanism. We therefore quantitated the in vivo levels of RNA transcripts originating from both promoters (P1 and P2) by RNase protection. RNA was harvested from cpxA- or Δcpx-mutant strains containing either a control plasmid, pBAD22, or a plasmid that overexpresses NlpE, pND18. Expression of the distal promoter (P2) is induced 10-fold by NlpE overproduction, whereas the proximal promoter (P1) is not induced (Fig. 3a). Induction of P2 was not observed in strains containing a cpx null mutation [Fig. 3a].

We then investigated the effect that various mutations in the cpx locus have on expression from P2. The amount of RNA produced from the P2 promoter was assayed by RNase protection in strains containing either the wild-type cpx operon, the defined cpxRA deletion, or one of two cpxA* mutations. As is shown in Figure 4a, strains containing cpxA* mutations produced from four- to sevenfold more of the transcript originating from P2 relative to the amount produced in the cpx+ parent strain. This difference is highly significant, as determined by standard t-test analysis.

Kinetics of activation of the Cpx pathway by NlpE overproduction

NlpE overproduction is routinely used as an activator of the Cpx pathway, but the mechanism underlying such activation is unknown. To gain further insight into the phenomenon, we determined the kinetics of activation of the Cpx pathway following induction of NlpE synthesis. A rapid activation of Cpx may reflect a more direct role for NlpE in activating the Cpx pathway. However, if the kinetics of activation of Cpx were found to be slow and the strength of the signal to increase with time of synthesis, NlpE overproduction may result in an indirect effect on cell envelope structure. NlpE synthesis was induced by the addition of arabinose to exponentially growing cultures, and the expression from the dsbA P2 promoter was measured at various times by RNase protection. The dsbA P2 transcript is rapidly and maximally induced within 5 min of induction of NlpE [Fig. 5]. Overproduction of another exported protein, alkaline phosphatase (AP), did not significantly induce dsbA transcription, despite the fact that AP was more highly expressed than NlpE in these experiments, as revealed by Coomassie staining of total proteins at the end of the experiment [data not shown]. These results are consistent with those of Danese et al. (1995) in which the overproduction of several other exported proteins did not stimulate the Cpx response and suggest that NlpE is a specific and potent activator of the Cpx pathway.

Binding of His<sub>s</sub>-CpxR and His<sub>s</sub>-CpxR-P proteins to the transcriptional regulatory region of the degP gene

To determine whether the CpxR protein, or more likely the phosphorylated form [CpxR-P], serves directly as a transcriptional regulator of the degP locus, we tested whether binding of the CpxR protein to DNA fragments containing the transcriptional regulatory region of the degP gene could be detected. For this study, a derivative of the CpxR protein [His<sub>s</sub>-CpxR] bearing an amino-terminal, oligohistidine extension was used prior to or after incubation with acetyl phosphate.

In a preliminary search for specific DNA-binding sites for the His<sub>s</sub>-CpxR and His<sub>s</sub>-CpxR-P proteins, electrophoretic mobility shift (EMS) and DNase I footprinting assays were performed using a 362-bp fragment (derived from pBTKSdegP1) that includes 301 bp of the region...
The Cpx cell envelope stress response

---

**Figure 4.** Expression from the \( \text{yihE-dsbA} \) P2 promoter and the \( \text{ppiA} \) P1 promoter in \( \text{cpx} \) mutants. Three independent cultures of each strain were grown to an OD\(_{600}\) of ~0.3. Samples were then taken, and expression of mRNA from each promoter was assayed by RNase protection. The average value for each strain (labeled 1-5) and the S.D. (error bars) for that average are expressed relative to the values obtained for strain 1. Results for the \( \text{yihE-dsbA} \) P2 promoter are shown in **a** (top) and those for the \( \text{ppiA} \) P1 promoter are shown in **b** (bottom). Strain 1 (JP406); strain 2 (ECL1212); strain 3 (JP408); strain 4 (JP467); strain 5 (JP466).

---

upstream of the initiation codon of \( \text{DegP} \). The results obtained were indicative of the presence of two distinct and well-separated binding sites, at which the phosphorylated form of the protein appeared to bind with a significantly higher affinity [data not shown].

Figure 6a shows DNase I footprinting analysis of the binding of the \( \text{His}_{\epsilon}\text{-CpxR} \) and \( \text{His}_{\epsilon}\text{-CpxR-P} \) proteins to a 249-bp fragment [derived from \( \text{pBTKSdegP2} \)] that includes the \( \sigma^\text{E} \) promoter of the \( \text{degP} \) gene and that contains the stronger of the two binding sites identified in the preliminary analysis. Clear DNase I footprints are only readily apparent with the \( \text{His}_{\epsilon}\text{-CpxR} \) protein at the highest concentration of protein employed (720 nm, lanes 12,24). In contrast, clear DNase I footprints are observed with the \( \text{His}_{\epsilon}\text{-CpxR-P} \) protein at a significantly lower concentration (180 nm, lanes 4,16). As the concentration of the \( \text{His}_{\epsilon}\text{-CpxR-P} \) protein is increased further, the regions of protection from DNase I cleavage expand in a unidirectional fashion to include sequences located downstream of the promoter.

Figure 6b shows a similar analysis of protein binding to a 213-bp fragment containing the weaker \( \text{His}_{\epsilon}\text{-CpxR-P} \) binding site, which is located upstream of the \( \sigma^\text{E} \) promoter–proximal site described above. In this case, DNase I footprints are not apparent with the \( \text{His}_{\epsilon}\text{-CpxR} \) protein even at the highest concentration of protein employed (720 nm, lanes 9,18). In contrast, clear DNase I footprints are observed on both strands of the template with the \( \text{His}_{\epsilon}\text{-CpxR-P} \) protein at a concentration of 360 nm (lanes 3,12).

The core \( \text{His}_{\epsilon}\text{-CpxR-P} \) footprints at both sites are indicated in Figure 6c (labeled as “\( \text{CpxR-P binding site I} \)” or “\( \text{CpxR-P binding site II} \)”]. Each site corresponds to the sequences at which DNase I cleavage is most significantly affected by \( \text{His}_{\epsilon}\text{-CpxR-P} \) protein binding [i.e., when \( \text{His}_{\epsilon}\text{-CpxR-P} \) is present at only relatively low concentrations].

**Binding of \( \text{His}_{\epsilon}\text{-CpxR} \) and \( \text{His}_{\epsilon}\text{-CpxR-P} \) proteins to the transcriptional regulatory region of the \( \text{yihE-dsbA} \) operon**

Preliminary studies suggested that the CpxR protein binds in a phosphorylation-stimulated manner to a

---

**Figure 5.** Kinetics of induction of the \( \text{yihE-dsbA} \) operon by NlpE. Strains containing the plasmids pND18 (solid bars, overexpresses NlpE), pDB3 (hatched bars, overexpresses AP), or pBAD22 (open bars, control plasmid) were grown aerobically in minimal maltose medium with ampicillin at 37°C. At time zero (corresponding to an OD\(_{600}\) of ~0.2), a sample from each culture was taken and then arabinose added to a final concentration of 0.2%. Samples were taken at 5, 10, 20, and 40 min after the addition of arabinose. \( \text{mRNA} \) originating from the P2 promoter of the \( \text{yihE-dsbA} \) operon was quantitated by RNase protection. The results are expressed relative to the values obtained for each culture at time zero. The strains were JP444 (\( \text{cpx}^+\text{pDB3} \)), JP445 (\( \text{cpx}^+\text{pBAD22} \)), and JP446 (\( \text{cpx}^+\text{pND18} \)). Similar results were obtained using the ara" strains JP472 and JP558.
Figure 6. CpxR-P binding at sites located in the region upstream of the \textit{degP} gene. 

[a] DNase I protection assays of the binding of His$_{6}$-CpxR and His$_{6}$-CpxR-P to \textit{degP} site I. A 249-bp DNA fragment (obtained by PCR amplification of the \textit{degP} promoter region of pBTKS\textit{degP2} with primers DEGP3 and DEGP2) was end-labeled with $^{32}$P at its 5' termini by treatment with T4 polynucleotide kinase, and uniquely labeled substrates were generated by EcoRI (lanes 1–12) or BamHI (lanes 13–24) digestion. The substrate DNA was present at -2 nM in reactions that also contained no added protein (lanes 1, 7, 13, 19), 36 nM His$_{6}$-CpxR-P (lanes 2, 14), 90 nM His$_{6}$-CpxR-P (lanes 3, 15), 180 nM His$_{6}$-CpxR-P (lanes 4, 16), 360 nM His$_{6}$-CpxR-P (lanes 5, 17), 720 nM His$_{6}$-CpxR-P (lanes 6, 18), 36 nM His$_{6}$-CpxR (lanes 8, 20), 90 nM His$_{6}$-CpxR (lanes 9, 21), 180 nM His$_{6}$-CpxR (lanes 10, 22), 360 nM His$_{6}$-CpxR (lanes 11, 23), or 720 nM His$_{6}$-CpxR (lanes 12, 24). The sequencing ladders were generated by extension of $^{32}$P-end-labeled DEGP3 primer for the coding strand or DEGP2 primer for the noncoding strand using pBTKS\textit{degP2} as a template. The core His$_{6}$-CpxR-P binding site I is indicated by the thick line; the thin line indicates the limits of the sequence shown in the lower half of c. 

[b] DNase I protection assays of the binding of His$_{6}$-CpxR and His$_{6}$-CpxR-P to \textit{degP} site II. A 213-bp DNA fragment (obtained by amplification of the \textit{degP} promoter region of pBTKS\textit{degP3} with primers DEGP4 and DEGP5) was end-labeled with $^{32}$P, and uniquely labeled substrates were generated by EcoRI (lanes 5–9) or BamHI (lanes 10–18) digestion. The substrate DNA was present at -2 nM in reactions that also contained no added protein (lanes 5, 14), 90 nM His$_{6}$-CpxR-P (lanes 2, 11), 180 nM His$_{6}$-CpxR-P (lanes 3, 12), 360 nM His$_{6}$-CpxR-P (lanes 4, 13), 90 nM His$_{6}$-CpxR (lanes 6, 15), 180 nM His$_{6}$-CpxR (lanes 7, 16), 360 nM His$_{6}$-CpxR (lanes 8, 17), or 720 nM His$_{6}$-CpxR (lanes 9, 18). Sequencing ladders were generated by extension of $^{32}$P-end-labeled DEGP4 primer for the coding strand or DEGP5 primer for the noncoding strand using pBTKS\textit{degP3} as a template. The core His$_{6}$-CpxR-P binding site II is indicated by the thick line; the thin line indicates the limits of the sequence shown in the upper half of c. 

[c] The location of CpxR-P binding sites in the region upstream of the \textit{degP} gene. Elements of the $\delta^k$ promoter (Erickson and Gross 1989) are indicated. The core CpxR-P binding sites I and II, as defined by the patterns of altered DNase I reactivity observed on both strands in the presence of a limiting concentration of His$_{6}$-CpxR-P, are indicated by the brackets.
single site in the transcriptional regulatory region of the yihE-dsbA operon. Figure 7a shows DNase I footprinting analysis of the binding of the His6-CpxR and His6-CpxR-P proteins to a 275-bp fragment [derived from pBTKSdsbA2] that includes the P2 promoter of the yihE-dsbA operon. DNase I footprints are readily apparent on the noncoding strand with the lowest concentration of His6-CpxR-P used [36 nM], whereas equivalent footprints are only observed with the His6-CpxR protein at the highest concentration of protein employed [720 nM]. As the concentration of the His6-CpxR-P protein is increased, the region of protection from DNase I cleavage expands bidirectionally. Significant differences in the apparent affinities of the His6-CpxR and His6-CpxR-P proteins are also indicated by the DNase I footprints obtained on the coding strand (lanes 1–12); a core His6-CpxR-P footprint is indicated in Figure 7b.

Computer-assisted alignments of the DNA sequences present in the three core footprints characterized to this point yielded only a pentamer motif (5’-GTAA(N)6GTAA-3’; indicated with arrows in Figs. 6c and 7b) as the most significant primary sequence element present in all three sequences. In the case of the yihE-dsbA site, it was noted that a second imperfect copy of the motif (5’-GTAAAG-3’) was present in close proximity with only 5 bp [or half a helical turn] located between them (also indicated in Fig. 7b). A computer search of the E. coli genomic DNA sequences present in GenBank [release 96.0] with the sequences 5'-GTAA(N)6GTAA-3' and 5'-GTAA(N)7GTAA-3' revealed the presence of a number of these elements, as would be expected given the relatively low complexity of the search motifs. However, subsequent analysis indicated that the presence of a subset of the motifs identified may be of physiological significance because they were present in known [or putative] transcriptional regulatory regions of genes encoding products related to protein folding.

Figure 7. [a] DNase I protection assays of the binding of His6-CpxR and His6-CpxR-P to the promoter of the yihE-dsbA operon. A 275-bp DNA fragment [obtained by amplification of the yihE-dsbA promoter region of pBTKSdsbA2 with primers DSBA3 and DSBA2] was end-labeled with 32P, and uniquely labeled substrates were generated by EcoRI [lanes 1–12] or BamHI [lanes 13–23] digestion. The substrate DNA was present at ~2 nM in reactions that also contained no added protein [lanes 1, 7, 13, 18], 36 nM His6-CpxR-P [lanes 8, 19], 90 nM His6-CpxR-P [lanes 9, 20], 180 nM His6-CpxR [lanes 10, 21], 360 nM His6-CpxR-P [lanes 11, 22], 720 nM His6-CpxR-P [lanes 12, 23], 36 nM His6-CpxR [lane 2], 90 nM His6-CpxR [lanes 3, 14], 180 nM His6-CpxR [lanes 4, 15], 360 nM His6-CpxR [lanes 5, 16], or 720 nM His6-CpxR [lanes 6, 17]. Sequencing ladders were generated by extension of 32P-end-labeled DSBA3 primer for the coding strand or DSBA2 primer for the noncoding strand using pBTKSdsbA2 as a template. The core His6-CpxR-P binding site is indicated by the thick line; the thin line indicates the limits of the sequence shown in b. [b] The location of CpxR-P binding sites in the promoter region of the yihE-dsbA operon. Elements of the putative σ70 promoter [Belin and Boquet 1994] are indicated; the core CpxR-P binding site is indicated by the brackets.
processing or maturation of proteins in the periplasm, it was of interest to determine whether the ppiA gene may also be a member of the Cpx regulon.

EMS and DNase I footprinting studies suggested that phosphorylated CpxR binds to a single site in the transcriptional regulatory region of the ppiA gene. Figure 8a shows DNase I footprinting analysis of the binding of the His6-CpxR and His6-CpxR-P proteins to a 267-bp fragment (derived from pBTKSppiA1) that includes the P1 promoter of the ppiA gene [Norregaard-Madsen et al. 1994]. Under the conditions used in this analysis, clear DNase I footprints are readily apparent on the noncoding strand with the lowest concentration of His6-CpxR-P (36 nM), whereas equivalent footprints are only observed with the highest concentration of unphosphorylated His6-CpxR (360 nM). As the concentration of the His6-CpxR-P protein is increased, the region of protection from DNase I cleavage again expands in a bidirectional fashion; a core His6-CpxR-P footprint is indicated in Figure 8b.

Cpx regulates transcription of the ppiA gene

If the CpxR-P binding site in the ppiA promoter region is of physiological significance, then changes in expression of the ppiA gene may be expected to be observed under conditions that activate the Cpx pathway. We therefore studied changes in the levels of RNA transcripts originating from the P1 promoter after induction of NlpE overexpression; the quantitated data is shown in Figure 3b. Expression from the ppiA P1 promoter is induced 2.5-fold when NlpE is overexpressed in strains containing a wild-type cpxRA operon but not in strains in which the cpx operon is deleted. This level of induction is lower than that seen for the yihE-dsbA operon, which was induced 5- to 10-fold in the same experiment.

To more definitively establish the role that the Cpx system plays in regulating the expression of the ppiA gene, we then examined the effect that various cpx mutations have on the expression of the ppiA P1 promoter. Three independent cultures of each strain containing either the wild-type cpx operon, the defined cpxRA null mutation, or one of two cpxA* mutations were grown at 37°C, and the in vivo level of expression of the mRNA originating from the P1 promoter determined by RNase protection. The quantitated results were averaged and are shown in Figure 4b. Two strains containing different cpxA* mutations both express significantly more (-2.5-fold; P < 0.01, n = 3) of the ppiA P1 transcript relative to the cpx÷ parent strain. In addition, we also reproducibly observed a two- to threefold decrease (P < 0.005, n = 3) in expression of the P1 promoter in the cpx null mutant.

Figure 8. (a) DNase I protection assays of the binding of His6-CpxR and His6-CpxR-P to the ppiA promoter region. A 267-bp DNA fragment (obtained by amplification of the ppiA region of pBTKSppiA1 with primers PPIA1 and PPIA2) was end-labeled with 32P and uniquely labeled substrates were generated by EcoRI (lanes 1-9) or BamHI (lanes 10-18) digestion. The substrate DNA was present at ~2 nM in reactions that also contained no added protein (lanes 1,10), 36 nM His6-CpxR-P (lanes 2,11), 90 nM His6-CpxR-P (lanes 3,12), 180 nM His6-CpxR-P (lanes 4,13), 360 nM His6-CpxR-P (lanes 5,14), 36 nM His6-CpxR (lanes 6,15), 90 nM His6-CpxR (lanes 7,16), 180 nM His6-CpxR (lanes 8,17), 360 nM His6-CpxR (lanes 9,18). Sequencing ladders were generated by extension of 32P-end-labeled PPIA1 primer for the coding strand or PPIA2 for the noncoding strand using pBTKSppiA1 as a template. The core His6-CpxR-P binding site is indicated by the thick line; the thin line indicates the limits of the sequence shown in b. (b) The location of CpxR-P binding sites in the σ70 P1 promoter region of the ppiA gene. Elements of the putative σ70 P1 promoter (Norregaard-Madsen et al. 1994; this paper) are indicated; the core CpxR-P binding site is indicated by the brackets.
The effects that cpx mutations have on the expression of the ppiA P1 promoter (Figs. 3b and 4b), taken together with results from DNase I footprinting (Fig. 8), suggest that the ppiA gene is a member of the Cpx regulon.

Discussion

New targets of the Cpx signal transduction pathway

Evidence presented here suggests that CpxA and CpxR directly regulate the expression of genes encoding periplasmic proteins required for protein folding and degradation. When the Cpx pathway is activated by cpxA* mutations or by NlpE overexpression, DsbA and DegP are coordinately induced. Elevated expression of DsbA is attributable to increased transcription of the P2 promoter of the yihE-dsbA operon. The phosphorylated form of CpxR binds with significantly higher affinity than the unphosphorylated form to specific sequences within the transcriptional regulatory regions of the degP gene, the ppiA gene, and the yihE-dsbA operon. Transcriptional activation of these genes is therefore likely to be mediated by phosphorylation of CpxR by CpxA.

Identification of a consensus sequence for CpxR binding

Analysis of the sequences of the degP and dsbA promoters to which the His-tagged CpxR-P protein bound resulted in the identification of a putative consensus binding site for CpxR at σ70 promoters of 5'-GTAANσ-7 GTAA-3'. Using this sequence, we identified the ppiA gene, encoding a periplasmic PPIase, as a potential regulatory target. The results of DNA binding and expression studies suggest that the phosphorylated form of the CpxR protein (CpxR-P) directly serves as a transcriptional activator of the P1 (σ70) promoter of the ppiA gene. However, Cpx-dependent induction of the ppiA transcript is lower than that observed for the dsbA or degP genes, and deletion of the cpx operon leads to a 2.5-fold decrease in ppiA P1 promoter expression. Hence, the Cpx system appears to contribute to the basal level of expression of the ppiA P1 promoter under normal growth conditions, suggesting that the unphosphorylated form of CpxR may be capable of binding to, and activating transcription from, the ppiA P1 promoter in vivo. Alternatively, a low level of the phosphorylated form of CpxR may be present in cells in “unstimulated” growth conditions. A similar conclusion can be drawn from the results of a previous study, as expression of the degP promoter was observed to be reproducibly lower in strains deleted for the cpx operon (Danese et al. 1995).

Mechanism of CpxR-mediated transcriptional activation

The results obtained are compatible with the notion that CpxR-P serves in a direct fashion as a transcriptional activator of the σ70 promoter of the degP locus. By analogy with observations on the mechanisms by which other prokaryotic transcriptional activators function (Busby and Ebright 1994), it seems likely that CpxR-P bound at the degP promoter–proximal binding site [site I] may facilitate recruitment of σ70-containing RNA polymerase holoenzyme by interacting with the carboxy-terminal domain of the σ-subunit. The upstream CpxR-P–binding site [site II] is absent in the Ψ(degP-lacZ) construct used in previous studies (Danese et al. 1995). Because we find no obvious discrepancies between our results obtained by examining the expression of DegP protein and those obtained using the degP–lacZ fusion, we cannot currently say how site II contributes to degP expression.

The location of the DNase I footprints in the ppiA and yihE-dsbA promoter regions suggests that transcriptional activation may be mediated by interaction of CpxR-P with the σ70 subunit of RNA polymerase holoenzyme, as is proposed for KcI protein-mediated activation at the ΨPRM promoter (Joung et al. 1994; Li et al. 1994), or via interaction with both the carboxy- and amino-terminal domains of the σ-subunit as proposed for the catalobite activator protein at class II promoters (Niu et al. 1996). Hence, CpxR-P appears able to activate transcription of promoters recognized by both σ70 [degP] and σ70 [ppiA and yihE-dsbA] containing RNA polymerase holoenzymes.

Are other periplasmic protein-folding catalysts members of the Cpx regulon?

The identification of additional genes containing the consensus CpxR-binding site may help to further elaborate the Cpx regulon. For example, the regulatory region of the surA gene, the expression of which was not affected by cpxA* mutations, does not contain the putative consensus sequence. Other genes such as dsbB, dsbC, and dsbD also lack obvious matches to the consensus sequence. However, a search of the E. coli genome database demonstrates that this sequence is located in the transcriptional regulatory regions of a number of potentially relevant genes, such as rpoE, rpoH, groE, and skp [ompHL]. As is shown in Figure 9, the 5'-GTAANσ-7 GTAA-3' motif is also present in the upstream regulatory region of the cpxRA operon, suggesting that the operon may be subject to positive feedback regulation.

Potential significance of the Cpx regulon

Several results suggest that increased expression of periplasmic folding catalysts effected by CpxA and CpxR may promote the folding or repair of cell envelope proteins. For example, overexpression of DsbA can suppress many of the phenotypes associated with a dsbB null mutation (Bardwell et al. 1993). Thus, although mutants deleted for the cpx operon are not defective in disulfide bond formation, it seems likely that under some conditions, DsbA induction by Cpx may promote disulfide
bond formation. Furthermore, in vitro experiments show that the presence of a PPIase can increase the efficiency of a disulfide bond isomerase (Schonbrunner and Schmid 1992). Hence, the PPIase PpiA, which appears to be coordinately regulated with DsbA by Cpx, may enhance the catalytic efficiency of DsbA or other as yet to be identified protein disulfide isomerases. Increased expression of DegP may be necessary to degrade proteins that are inappropriately folded or which cannot be repaired following oxidative (or other) damage.

**Does Cpx play a role in virulence?**

The Cpx system may come into play when the cell experiences environmental conditions that affect protein folding, such as those that are thought to be encountered by certain *enterobacteriaceae* in host tissues during infection. For example, a variety of immune cells produce reactive oxygen species \( \text{NO, } \text{O}_2^- \) that can cause significant oxidative damage to proteins (Badwey and Karnovsky 1980; Nathan and Hibbs 1991; Babior 1992; Marletta 1993). Several studies have demonstrated that DegP is required for normal resistance to reactive oxygen species and is necessary for both survival in macrophages and full virulence in mice (Johnson et al. 1991; Baumler et al. 1994; Elzer et al. 1994). Hence, it seems possible that the Cpx-dependent expression of DegP may contribute to the protection of the cell from the damaging effects of free radical oxygen species by proteolytically degrading damaged proteins in the cell envelope (Boucher et al. 1996; Govan and Deretic 1996). It will be of interest to ascertain if free radical damage of cell envelope proteins is one of the stimuli that leads to activation of the CpxRA system.

Mutations in some of the other genes of the Cpx regulon also result in attenuation of pathogenesis in vivo. In *Shigella sonnei*, the Cpx system regulates expression of the *virF* gene in response to pH changes (Nakayama and Watanabe 1995). VirF is a transcriptional activator that positively regulates expression of the *IpaB*, *IpaC*, and *IpaD* proteins, which are important for invasion of epithelial cells (Menard et al. 1996). Expression and proper folding of these proteins requires DsbA, and *S. flexneri* *dsbA* mutants are attenuated for invasion (Watarai et al. 1995). A DsbA homolog in *Vibrio cholera* is needed for the maturation of secreted virulence factors (Peek and Taylor 1992). It therefore seems possible that the Cpx system also regulates a variety of genes encoding products that are not directly involved in processes related to
The Cpx cell envelope stress response

protein folding but that play important roles during pathogenesis.

Relationship between the Cpx and σE pathways

These findings and how they relate to results in the accompanying paper [Danese and Silhavy, this issue] are summarized in Figure 10. E. coli contains at least two periplasmic stress response systems, the Cpx pathway and the σE pathway. The σE pathway is induced by conditions that lead to misfolding or misassembly of outer membrane proteins (Omps), such as overexpression of Omps or inactivation of the surA gene [Mecsas et al. 1993; Rouviere et al. 1995]. Stress signals from the cell envelope are communicated to σE by the Rse proteins [De Las Peñas 1997]. In response to these stresses, σE transcribes the degP gene and a gene encoding another periplasmic PPIase, fkpA (see Danese and Silhavy, this issue). The Cpx pathway responds to cell envelope stresses such as changes in pH or NlpE overexpression and activates the transcription of genes transcribed from both σ70 and σE promoters [Danese et al. 1995; Nakayama and Watanabe 1995; Danese and Silhavy, this issue]. The Cpx regulon includes at least dsbA, degP, and ppiA and possibly also virF and cpxRA, all of which contain sequences similar to the CpxR consensus sequence (solid boxes in Fig. 10) upstream of their promoters. The Cpx and σE pathways, which appear to respond to different stresses and generally regulate different genes, overlap to control the expression of the degP gene. The presence of a putative CpxR-binding site in the regulatory regions of several members of the σE regulon may indicate that the Cpx and σE regulons form a network of overlapping and possibly closely integrated regulatory pathways.

Materials and methods

Bacterial strains

The relevant genotypes of the bacterial strains used in this study are listed in Table 1. Three different cpxA* alleles were used in this study. The cpxA9 mutant is temperature sensitive, exhibiting cpxA* phenotypes at 42°C but not at 30°C. The other two mutants, cpxA2.1 and cpxA9.4, are phenotypically more severe than the cpxA9 mutant and exhibit cpxA* phenotypes at both 23°C and 42°C.

Immunoprecipitation of radiolabeled proteins

The rates of synthesis of DsbA, DegP, SurA, MBP, and OmpA were determined by pulse-chase immunoprecipitation experiments. Strains were grown at the indicated temperatures in M63 medium containing 0.2% maltose supplemented with 18 amino acids (50 μg/ml each). Cultures were given a 20-sec pulse of [35S]methionine (100 μCi/ml) and chased with excess, unlabeled methionine. Samples were taken after 1 min of chase, and the labelings were stopped on ice. Immunoprecipitation of radiolabeled proteins was carried out essentially as described [Pogliano and Beckwith 1993]. Rabbit polyclonal antibodies specific for each protein, DsbA (Bardwell et al. 1993), DegP, SurA (a gift of S. Lazar and R. Kolter, Harvard Medical School, Boston, MA), and OmpA were used for immunoprecipitations. DegP, SurA, MBP, and OmpA were separated by 10% SDS-PAGE. DsbA and OmpA were separated by 12.5% SDS-PAGE. The counts in each radioactive band were quantitated using a Molecular Dynamics PhosphorImager. Counts in the DegP and DsbA bands were normalized to OmpA as an internal control.

Table 1. Strains and plasmids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL525</td>
<td>MC4100 Δ[argF-lac][U169 araD139]</td>
<td>Iuchi and Lin (1988)</td>
</tr>
<tr>
<td>ECL3000</td>
<td>MC4100 Δ[pha-cpxRA-pfkA]15 zig-1::Tnl10</td>
<td>A.S. Lynch and E.C.C. Lin (unpubl.)</td>
</tr>
<tr>
<td>JP406</td>
<td>ECL525 F'pOXgen</td>
<td>J. Dong, J. Pogliano, and E.C.C. Lin (unpubl.)</td>
</tr>
<tr>
<td>JP408</td>
<td>JP406 Δ[cpxRA]</td>
<td>this study</td>
</tr>
<tr>
<td>JP406</td>
<td>ECL1212/1216/1215</td>
<td>this study</td>
</tr>
<tr>
<td>JP413</td>
<td>JP13/pDB3</td>
<td>this study</td>
</tr>
<tr>
<td>JP444</td>
<td>JP413/pBAD22</td>
<td>this study</td>
</tr>
<tr>
<td>JP446</td>
<td>JP413/pND18</td>
<td>this study</td>
</tr>
<tr>
<td>JP466</td>
<td>JP406 argE::Tnl10</td>
<td>this study</td>
</tr>
<tr>
<td>JP467</td>
<td>JP406 argE::Tnl10 cpxA2.1</td>
<td>this study</td>
</tr>
<tr>
<td>JP468</td>
<td>JP406 argE::Tnl10 cpxA9.4</td>
<td>this study</td>
</tr>
<tr>
<td>ECL1215</td>
<td>JP406 ara*</td>
<td>this study</td>
</tr>
<tr>
<td>ECL1216</td>
<td>ECL1212 ara*</td>
<td>this study</td>
</tr>
<tr>
<td>ECL472</td>
<td>ECL1215/pBAD22</td>
<td>this study</td>
</tr>
<tr>
<td>ECL474</td>
<td>ECL1216/pBAD22</td>
<td>this study</td>
</tr>
<tr>
<td>ECL558</td>
<td>ECL1215/pND18</td>
<td>this study</td>
</tr>
<tr>
<td>ECL560</td>
<td>ECL1216/pND18</td>
<td>this study</td>
</tr>
<tr>
<td>Plasmid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pBAD22</td>
<td>bla, pBR ori, Phad promoter</td>
<td>Guzman et al. (1995)</td>
</tr>
<tr>
<td>pND18</td>
<td>overexpresses Nlpe</td>
<td>Danese et al. (1995)</td>
</tr>
<tr>
<td>pDB3</td>
<td>overexpresses alkaline phosphatase</td>
<td>Guzman et al. (1995)</td>
</tr>
</tbody>
</table>
and expressed in arbitrary units. OmpA is expressed constitutively at high levels, and its expression is not affected by the conditions used here; similar results were obtained if DegP or DsbA expression was normalized to the culture OD600 at the time of sampling. To ensure that the DegP protein "unun precipitated in these experiments is encoded by the cpxA9 degP::Tn5 mutant double mutant was also examined; no protein that migrates at the position of DegP was seen in this strain (data not shown).

Plasmid constructions
Plasmids pQE30 and pREP4 were obtained from Qiagen Ltd. To create pQE30CpxR, a 700-bp region of DNA containing the cpxR open reading frame (positions 1246–1946 in GenBank file L14579) was amplified by PCR with primers CPXR5 (5'-CCCGGATCCCATATGAATAAAATCCTGTTAGTTGATG-3') and CPXR3 (5'-CCCGGATCCCTGCACTTATCATGAGCAGAAACCATCAG-3') using E. coli MC4100 DNA as a template. The PCR product was digested with BamHI and PsiI, and the resulting 710-bp fragment was cloned between the corresponding sites of pQE30. pQE30cpxR, expresses a CpxR fusion protein, composed of full-length CpxR with an amino-terminal Met-Arg-Gly-Ser-[35S]methionine at the time of RNA isolation, and the lysates were immunoprecipitated with appropriate antibodies as described (Bost and Belin 1995). Culture samples (2 ml) were lysed by boiling for 2 min after addition of 0.2 ml of 0.5 M Tris-HCl (pH 8.1), 0.4% SDS, and 20 mM EDTA (Belin et al. 1979). The lysates were made 0.2 M with NaOAc (pH 4.7), extracted twice with phenol/chloroform, and precipitated with ethanol after digestion of one-sixth volume of glacial acetic acid. Nucleic acids were resuspended in 10 mM Tris-HCl (pH 7.5), 20 mM NaCl, and 5 mM MgOAc, digested for 20 min at 37°C with 30 units of RNase-free DNase (Promega), extracted with phenol/chloroform, and precipitated with ethanol. The RNA yield was ~50 µg of cells (As30); the integrity of the RNAs was verified by electrophoresis.

RNA isolation
Cells were grown in M63 medium supplemented with 18 amino acids (50 µg/ml each), 0.2% maltose and 0.4% glycerol, and induced with 0.2% arabinose for the indicated times. Plasmid-bearing cells were grown in medium supplemented with 100 µg/ml of ampicillin. Aliquots were labeled for 30 sec with [35S]methionine at the time of RNA isolation, and the lysates were immunoprecipitated with appropriate antibodies as described (Bost and Belin 1995). Culture samples (2 ml) were lysed by boiling for 2 min after addition of 0.2 ml of 0.5 M Tris-HCl (pH 8.1), 0.4% SDS, and 20 mM EDTA (Belin et al. 1979). The lysates were made 0.2 M with NaOAc (pH 4.7), extracted twice with phenol/chloroform, and precipitated with ethanol after digestion of one-sixth volume of glacial acetic acid. Nucleic acids were resuspended in 10 mM Tris-HCl (pH 7.5), 20 mM NaCl, and 5 mM MgOAc, digested for 20 min at 37°C with 30 units of RNase-free DNase (Promega), extracted with phenol/chloroform, and precipitated with ethanol. The RNA yield was ~50 µg of cells (As30); the integrity of the RNAs was verified by electrophoresis.

RNase protection
The preparation of probes, hybridization, digestion of the unhybridized RNAs with RNase A, and analysis of the protected fragments were performed as described (Belin 1996a,b). The probes were labeled with [32P]UTP in the presence of 100 µM unlabeled UTP. For each sample, 2 µg of cellular RNA was hybridized to 4 ng of cRNA probe.

DNA-binding studies
Purification of the His6-CpxR protein from IPTG-induced E. coli ECL3000cells transformed with pREP4 and pQE30CpxR was performed as described previously for a similarly tagged protein (His6-CpxR) of the two-component response regulator family (Lynch and Lin 1996). On average, 2.5 mg of purified His6-CpxR was obtained from 1 gram of cell paste. Phosphorylation of the protein for use in DNA-binding studies used a standard reaction in which His6-CpxR (100 µg/ml of final concentration) was incubated for 1 hr at 30°C in a buffer containing 100 mM Tris-HCl (pH 7.4), 10 mM MgCl2, 125 mM KC1, and 50 mM acetyl phosphate (lithium, potassium salt from Sigma). In parallel, similar reactions lacking acetyl phosphate were used to prepare His6-CpxR for use in DNA-binding studies. The conditions used for in vitro DNA-binding assays by EMS and DNase I footprinting assays were as described previously (Lynch and Lin 1996).

Acknowledgments
We are grateful to Pete Yorgey for helpful discussions, Paul Danese and Tom Silhavy for communicating results prior to pPB3224 cloned between the BamHI and HindIII sites of pSP65; the DNA was linearized with Hpal and transcribed with SP6 RNA polymerase. The 414-nucleotide cRNA yields a 310-nucleotide protected fragment after hybridization to dsbA mRNA initiated at the downstream, P1 promoter. A PCR-amplified ppiA DNA fragment (positions 273–697; Kawamukai et al. 1989, GenBank accession no. M28363) was restricted with EcoRI and PvuII and subcloned between the EcoRI and Smul sites of pBSKS. Plasmid DNA was digested with Dral and transcribed with T7 RNA polymerase. The 355-nucleotide cRNA yields a 147-nucleotide protected fragment after hybridization to ppiA mRNA initiated at the P1 promoter (Norregaard-Madsen et al. 1994).

We are grateful to Pete Yorgey for helpful discussions, Paul Danese and Tom Silhavy for communicating results prior to pPB3224 cloned between the BamHI and HindIII sites of pSP65; the DNA was linearized with Hpal and transcribed with SP6 RNA polymerase. The 414-nucleotide cRNA yields a 310-
publication, Phil Silverman for the gift of strains containing the cpxA9 mutation, Dana Boyd for help with DNA sequence database analysis, and Kit Pogliano for critical reading of the manuscript. D.B. thanks Filo Silva for expert technical help. This work was supported by National Institutes of Health grants to J.B. and E.C.C.L. [GM40993 and GM39693]. D.B. was supported by the Fonds National Suisse de la Recherche Scientifique. J.B. is an American Cancer Society Research Professor.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References


Kawamura, M., H. Matsuda, W. Fuji, R. Utsumi, and T. Ko-
Pogliano et al.


Regulation of Escherichia coli cell envelope proteins involved in protein folding and degradation by the Cpx two-component system.


*Genes Dev.* 1997, 11:
Access the most recent version at doi:10.1101/gad.11.9.1169