Presence of the Cpx system in bacteria

The CpxA/R two-component signal transduction system is involved in the sensing of (via CpxA) and adaptation to (via CpxR in phosphorylated form) envelope protein distress in Escherichia coli (1, 9). The Cpx system has also been implicated in host-cell invasion and virulence. In uropathogenic E. coli, the Cpx pathway seems critical for the expression and assembly of P-pili (4, 5). In Shigella sonnei, CpxR-P directly activates the synthesis of VirF, the master regulator of virulence expression (7, 8). In Salmonella typhi, a cpxA::TnphoA mutant proved unable to invade human small intestinal epithelial cell cultures (6). Studies on the transcriptional regulation of cpxRA in non-pathogenic E. coli have yielded insights that may be relevant to related pathogens. CpxR-P autoactivates cpxRA transcription in concert with RpoS and an unknown activator at the onset of stationary-phase growth. In addition, Cpx signalling is feedback-inhibited by CpxP, a periplasmic protein which is under positive control of CpxR-P (2, 10).

Because of the importance of the Cpx pathway in stress adaptation and pathogenicity, we explored further the presence and distribution of cpxR, cpxA and cpxP in other prokaryotic genomes (http://wit.mcs.anl.gov/CGI; http://www.sanger.ac.uk/Projects/Microbes; http://www.tigr.org/tdb/CMR/) using the E. coli gene and protein sequences as references (default search settings). Among the genomes analysed (Archaeoglobus fulgidus; ‘Aquifex aeolicus’, Bacillus subtilis; Bordetella bronchiseptica, B. parapertussis, B. pertussis; Borrelia burgdorferi; Caulobacter crescentus; Campylobacter jejuni; Chlamydia trachomatis; Chlorobium tepidum; Clostridium acetobutylicum, C. difficile; Coxiella burnetii; Deinococcus radiodurans; Enterococcus faecalis; Haemophilus influenzae Rd; Helicobacter pylori; Klebsiella pneumonias; Methanobacterium thermoautotrophicum; Methanococcus jannaschii; Mycobacterium avium, M. bovis, M. leprae, M. tuberculosis; Mycoplasma genitalium; Neisseria meningitidis; Porphyromonas gingivalis; Pseudomonas aeruginosa, P. putida; ‘Pyrococcus horikoshii’; Rhodobacter capsulatus, R. sphaeroides; Salmonella typhi; Shewanella putrefaciens; Staphylococcus aureus; Streptococcus pneumoniae; Streptomyces coelicolor; Vibrio cholerae; Yersinia pestis), cpxR, cpxA and cpxP were found only in Salmonella typhi and Yersinia pestis.

CpxA, CpxR and CpxP of E. coli and S. typhi are 100, 100 and 88% identical, respectively (86, 87 and 88% identical at the nucleotide level). The divergence of the two CpxP proteins may indicate that they interact with additional periplasmic elements (10) that differ in these strains. The cpxRA promoter region of S. typhi (146 bp cpxRA/cpxP intergenic sequence) shares 95% nucleotide identity with that of E. coli. The sequence and position of the two putative CpxR-P recognition sites in the cpxRA promoter region are identical in E. coli and S. typhi (Table 1). The high degree of similarity of the CpxR/A/P protein sequences of the two species and the conserved CpxR-P recognition boxes in cpxRA, strongly suggest that, in S. typhi also, cpxRA is autogenously regulated and that the pathway is feedback-inhibited by CpxP. The presence of CpxR-P boxes in promoter regions of S. typhi target operons whose analogues are CpxR-P-controlled in E. coli (Table 1) further strengthens the notion that the Cpx system plays a similar role in both species.

<table>
<thead>
<tr>
<th>Operon</th>
<th>E. coli</th>
<th>S. typhi</th>
<th>Y. pestis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpxRA</td>
<td>57→70, 77→90</td>
<td>57→70, 77→90</td>
<td>56→69, 97→110</td>
</tr>
<tr>
<td>cpxP</td>
<td>57→70, 77→90</td>
<td>58→71, 78→91</td>
<td>60→73, 101→114</td>
</tr>
<tr>
<td>motABcheAW</td>
<td>194→207</td>
<td>195→208</td>
<td>272→285</td>
</tr>
<tr>
<td>isr</td>
<td>248→261</td>
<td>238→251</td>
<td>208→272</td>
</tr>
<tr>
<td>ppiA</td>
<td>79→92</td>
<td>76→89</td>
<td>80→93</td>
</tr>
<tr>
<td>ppiD</td>
<td>207→220</td>
<td>326→339</td>
<td>316→329</td>
</tr>
<tr>
<td>yihE-dsbA</td>
<td>68→81</td>
<td>67→80</td>
<td>–</td>
</tr>
<tr>
<td>degP</td>
<td>255→268</td>
<td>251→264</td>
<td>325→338</td>
</tr>
</tbody>
</table>

Table 1. Position of CpxR-P-binding sites (bp upstream from the start codon) in CpxR-P target operons of E. coli, S. typhi and Y. pestis.

*— indicates absence of a CpxR-P recognition box.

GUIDELINES
Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of Microbiology do not necessarily agree with the views expressed in Microbiology Comment.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.
The divergently transcribed cpxRA/cpxP intergenic region of Y. pestis shares only 55% nucleotide sequence identity with that of E. coli. Except for two extra sequence stretches (21 and 3 bp) in Y. pestis, this intergenic region shows 63% identity. In the Y. pestis promoter region, one CpxR-P recognition box is found at the same distance from the CpxR start codon as in E. coli, whereas the other box lies 21 bp further upstream of the start codon (Table 1). Y. pestis CpxA, CpxR and CpxP are 82, 88 and 47% identical, respectively, to those of E. coli. The surprising divergence of Y. pestis CpxP within the first 50 N-terminal residues (includes additional stretches) suggests that the C-terminal domain is the important one for interaction with the highly conserved CpxA. Additional CpxR-P recognition boxes were found in promoter regions of many, but not all, ORFs that are known to be CpxR-P-controlled in E. coli (Table 1). These findings imply that the Cpx pathways in the two bacterial species have evolved different modes of response to similar stresses.

In non-virulent Haemophilus influenzae Rd strain KW20, a CpxR-like coding sequence seems to be present (H10837). No CpxA or CpxP homologues were found. The putative CpxR is 6 aa shorter (internal) than CpxR of E. coli and shares with it only 58% amino acid identity. However, the two α-helix domains of H. influenzae CpxR share an amino acid identity of 87 and 80%, respectively, with the corresponding domains of E. coli CpxR, suggesting that the H. influenzae regulator might recognize the same box in target operon targets. Of the genes that are CpxR-P-controlled in E. coli, only ppiA and a degP homologue (HW1259; 53% identity in the 1358 bp overlap with E. coli degP) are present in H. influenzae. A CpxR-P box is present in the ppiA promoter region (79–92 bp upstream from the start codon) in the same position as in E. coli. However, no box is present in the promoter region of the degP homologue. Importantly, the cpxR promoter region of H. influenzae itself has no detectable similarity to that of E. coli, S. typhi or Y. pestis and does not contain a CpxR-P box, indicating that the expression of the cpxR homologue (H10837) is regulated differently. It thus seems possible that H. influenzae Rd strain KW20 has evolved to use the response regulator in a particular way. The absence of loci present in virulent H. influenzae isolates, such as pathogenicity islands and genes involved in capsule and fimbriae formation (3), implies that CpxA is yet another missing virulence factor in strain KW20. The only known natural niche for H. influenzae is within the human host, primarily in the upper respiratory tract. Therefore, this species may not require the ability to sense a transition from the free environment to the host milieu. If sensing that transition defines the role of CpxA/R, then Haemophilus would not require the typical CpxA/R pathway.

**Peter De Wulf, Brian J. Akerley and E. C. C. Lin**

Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA

---