Activation and Deletion of the CpxRA system Reduces the Virulence of Uropathogenic *Escherichia coli* in mice

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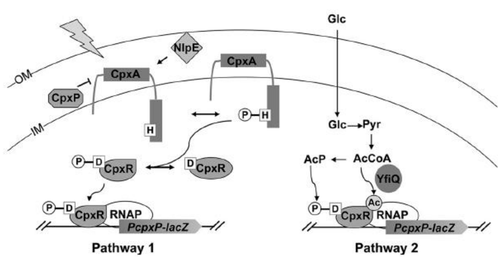
Abstract

CpxRA is an envelope stress response system that is found in uropathogenic Escherichia coli (UPEC) .CpxA is a sensor kinase/phosphatase that controls the activity of its response regulator CpxR. CpxRA functions in repairing the membrane and reducing protein traffic across the envelope; thus, activation of the CpxRA system downregulates the expression of multiple secreted factors, including virulence determinants. CpxRA activating mutants in *Haemophilus ducreyi* and *Salmonella typhimurium* are avirulent in human and murine models of infection, making CpxRA an attractive antivirulence target. In this study, we investigated the role of the CpxRA system in the virulence of UPEC. In competition experiments, both *cpxA* (system active) and *cpxR* (system inactive) deletion mutants were attenuated compared to wild type (WT) UPEC in a murine model of urinary tract infection. In a transcriptome analysis, 516 genes were differentially regulated between the *cpxA* and *cpxR* mutants. Of these, 310 were downregulated in the *cpxA* mutant compared to the *cpxR* mutant; these encoded proteins such as FimH that function in adhesion and are essential for murine infection. Compared to the WT, activation of the CpxRA system resulted in the differential regulation of 434 genes, 410 of those overlapped with those that were differentially regulated between the *cpxR* and the *cpxA* mutants. Only 11 genes were differentially regulated between the WT and the *cpxR* mutant, indicating that the CpxRA system is minimally active in the WT. These results suggest that activation of the CpxRA system results in attenuation of virulence of UPEC, likely due to the downregulation of essential virulence factors. In addition, an intact CpxRA system is also required for UPEC to establish infection, suggesting that the ability to respond to membrane stress is important in UPEC pathogenesis.

Background

- CpxRA is a two-component envelop stress response system that is found in UPEC and other *Enterobacteriaceae*.
- CpxA is a histidine kinase and a phosphatase, and CpxR is a response regulator.
- Upon sensing envelope stress, CpxA autophosphorylates at a conserved histidine residue and donates a phosphate group to a conserved aspartic acid residue on CpxR.
- Phosphorylated CpxR (CpxR-P) acts as a transcription factor and regulates the transcription of approximately 100 genes; genes that maintain envelope integrity are upregulated, whereas genes that encode secreted factors are downregulated.
- In the absence of membrane stress, CpxA functions as a phosphatase, rendering CpxR inactive.
- When wild-type cells are grown in minimal medium containing excess glucose, CpxR accepts phosphoryl groups from small-molecule donors, such as acetyl phosphate, and becomes activated.
- A *cpxA* deletion mutant (Δ*cpxA*) lacks the phosphatase activity and accumulates CpxR-P, resulting in activation of the CpxRA system.
- Deletion of *cpxR* (Δ*cpxR* mutant) results in an inactive CpxRA system.



van Rensburg et al. 2015, Antimicrobial Agents and Chemotheraphy

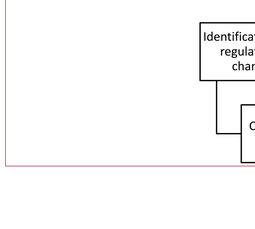
Activation of CpxRA cripples the virulence of multiple pathogens

- *An H. ducreyi* Δ*cpxA* mutant is unable to infect human volunteers; activation downregulates 7 virulence determinants, each of which is required for human infection.

  Spinola SM *et al.* **2010** *Infect and Immun*. 78: 3898-3904
  Gangaiah D *et al.* **2013** *J Bacteriol* 195: 3486-3502

- Mice inoculated with lethal doses of *S.* Typhimurium Δ*cpxA* mutant were not infected.

  Humphreys S *et al.* **2004** *Infect and Immun*. 72: 4654-4661



N. gonorrhoeae Δ*cpxA* mutant is outcompeted by wild type in murine vaginal model

Spinola and Jerse, unpublished data

Hypothesis

We hypothesize that the CpxRA system regulates essential virulence factors in UPEC.

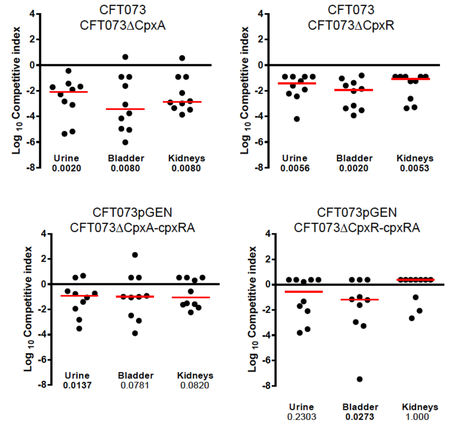
Methods

Murine ascending UTI model:
CBA/J mice were injected intravesically with 10^8 CFU of WT and either the Δ*cpxA* mutant or the Δ*cpxR* mutant (equal number of WT and mutant strain). The mice were euthanized 48 hours later. Bacterial loads of each strain were calculated from the urine, bladder, and kidneys. Competitive indices were calculated between each mutant strain and the WT strain. The experiments were repeated using mutant strains that were complemented with *cpxRA* in trans.

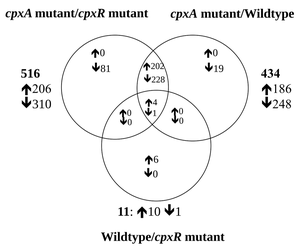
RNA-Seq:

Results

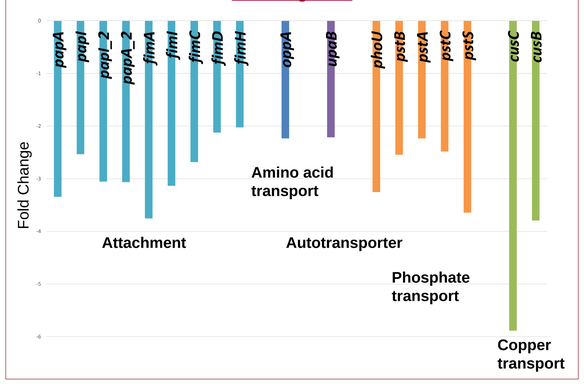
Activation and deletion of CpxRA attenuate the virulence of UPEC in mice



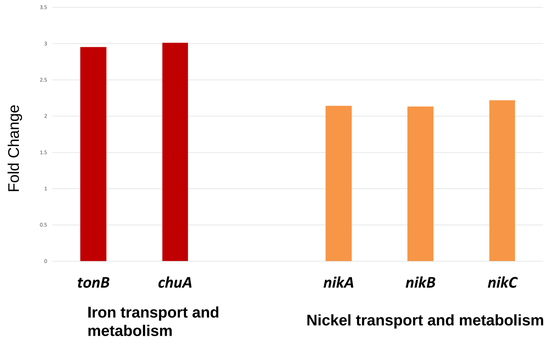
CpxRA is not active in the WT strain but is activated by deletion of *cpxA* in vitro



Activation of CpxRA downregulates many UPEC virulence & fitness genes



Activation of CpxRA upregulates some UPEC virulence & fitness genes



Summary

Activation of CpxRA severely compromises UPEC fitness, likely due to the downregulation of essential envelope and virulence genes. Loss of CpxR also results in attenuation of the virulence of UPEC, perhaps due to reduction in essential envelope repair functions that are required during times of stress encountered during infection.

Future directions

- Perform phenotypic assays to evaluate the effect of the CpxRA system on the adherence of UPEC to uroepithelial cells, and on iron acquisition.
- Compounds that activate the CpxRA system have been developed by our lab and will be tested for their ability to treat UPEC infection in mice.

Acknowledgments

This work was supported by the Arthur White Fellowship Fund, the Indiana Clinical and Translational Sciences Institute (CTSI), and was funded in part by a Project Development Teams (PDT) pilot grant (TR000006) and the IUPUI Funding Opportunities for Research Commercialization and Economic Success (FORCES).

References

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