Vibrio cholerae colonization of the host requires stringent response activation of TCP, the Toxin-Coregulated Pilus

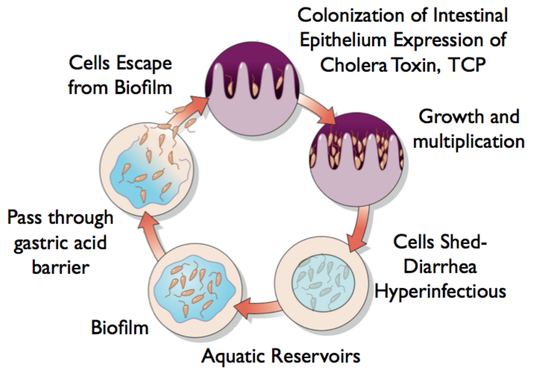
Arunima Mishra[1], Huajun He[1], Swathi Mall[1], Jennifer N. Cooper[1] and David M. Raskin[2]

[1]*The Methodist Hospital Research Institute, Houston, TX* [2]*Marian University College of Osteopathic Medicine, Indianapolis, IN 46222*

Abstract

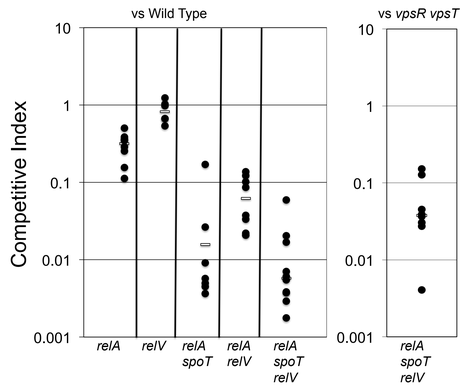
Cholera is an epidemic diarrheal disease caused by the gram-negative bacterium *Vibrio cholerae*. In order to colonize a host, *V. cholerae* must express the toxin-coregulated pilus (TCP), and the virulence factor that is primarily associated with diarrhea is cholera toxin (CT). CT and TCP expression is regulated by the ToxR regulon. Components of the ToxR regulon include ToxT, a transcription factor that directly initiates transcription of the CT and TCP genes. Upstream regulators include the integral membrane proteins TcpP and ToxR, transcription factors necessary for expression of *toxT*. TcpP and ToxR require TcpH and ToxS, respectively for their activities. Additional factors regulate expression of the upstream regulators. ToxR may also directly initiate transcription of the CT genes independently of ToxT. It is thought that TCP is required early in infection to colonize the small intestine and that CT is expressed later on to produce diarrhea and escape the host. It is not fully understood how the differential timing of CT and TCP expression occur due to their both being regulated by the same factors. We found that stringent response, the low nutrient stress response, was necessary for colonization of the infant mouse small intestine. We made deletions in the genes that regulate the stringent response and tested stringent response-defective mutants for expression of *ctxA* and *tcpA*, genes coding for components of CT and TCP respectively. Stringent response-defective strains had greatly reduced expression of *tcpA* and a small but significant reduction in expression of *ctxA*. We tested whether stringent response regulation of CT and TCP occurred through the ToxR regulon. We found that stringent response induced *toxT* and *tcpPH* expression, while repressing *toxRS*. The gene expression data was consistent with the requirement for stringent response in colonization of the mouse. Stringent response is required for TCP expression, with TCP required for normal colonization. The gene expression data showing that stringent response had opposite effects on different components in the ToxR regulon may explain how CT and TCP may be differentially regulated despite being controlled by the same transcriptional activators. We are continuing to investigate how stringent response affects the ToxR regulon and how differential regulation of CT and TCP occurs.

Vibrio cholerae life cycle



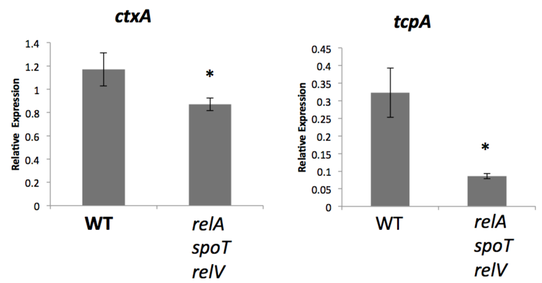
V. cholerae are exposed to different environments, from low nutrient aquatic environments to the high nutrient environment of the GI tract. We hypothesized that the low nutrient stress response-the stringent response-plays a role in regulating *V. cholerae* virulence factors. The genes involved in stringent response regulation-*relA spoT relV*-were knocked out and a stringent response defective strain was compared to a wild type strain (N16961) for regulation of virulence genes under different environmental conditions.

Role of Stringent Response in Colonization of Infant Mice



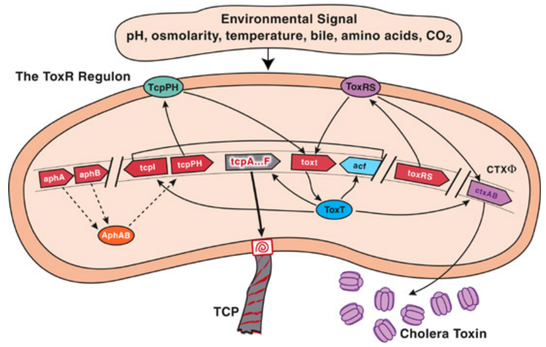
We found that stringent response defective strains were less able to colonize mice than wild type strains. Previous work has shown that stringent response regulates biofilm formation (He et al., 2012) and that biofilm formation is important for colonization of the small intestine (Zhu et al., 2003), so we compared the ability of stringent response defective strains to colonize mice to mutants unable to form biofilm (*vpsR vpsT* mutants). We found that stringent response mutants were unable to colonize mice even controlling for loss of biofilm.

Stringent Response is Necessary for Production of Cholera Toxin and TCP



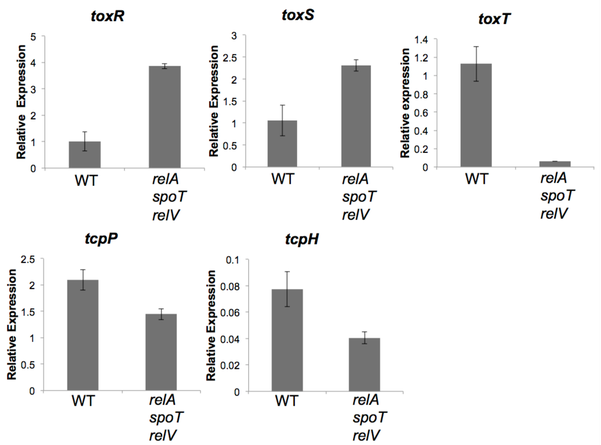
Cholera toxin (CT) is the primary factor necessary for production of diarhhea. The toxin co-regulated pilus (TCP) is one of the major factors responsible for colonization of the small intestine. Using qRT-PCR, we compared expression of CT and TCP in WT and in stringent response defective strains in AKI conditions-conditions in which *V. cholerae* produce virulence factors and may mimic what is seen in the host. We used the *ctxA* and *tcpA* genes as indicators, the first gene in the operons necessary for production of both factors. We found that stringent response was necessary for full expression of both factors, but more important for expression of TCP.

The ToxR Regulon



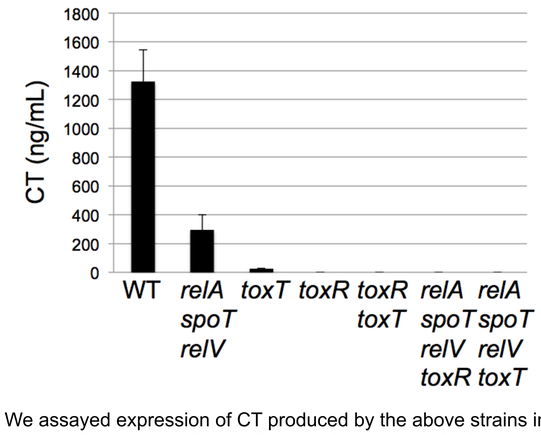
While it is regulation of *V. cholerae* virulence factors is not completely understood, a complex regulatory apparatus has been identified and termed the ToxR regulon. The CT genes (*ctxAB*) and the TCP genes (*tcpA-F*) and the upstreame regulators are indicated.

Stringent Response Regulation of the ToxR Regulon



We compared expression of ToxR regulon genes in WT and stringent response defective strains in AKI conditions. We found that stringent response repressed expression of *toxRS* and induced expression of *tcpPH* and *toxT*. All of these factors are necessary for full expression of both CT and TCP. That stringent response can both induce and repress ToxR regulon components is consistent with stringent response producing different expression patterns for CT compared to TCP. TCP expression is driven directly by ToxT, but CT expression is driven by both ToxT and ToxR.

Stringent Response Regulates CT Production Through Two Regulators



We assayed expression of CT produced by the above strains in AKI conditions using ELISA. The results are consistent with the gene expression studies. CT was produced by WT strain due to full expression of the ToxR regulon. The stringent response defective strain produced less CT due to loss of expression of *toxT*, but ToxR could still induce expression, especially with higher levels of *toxR* expression. The *toxT* mutant had lower levels of CT due to loss of ToxT direct induction in combination with stringent response inhibition of *toxR*. Deleting toxR or any combination of toxR, toxT and the stringent response genes (relA spoT relV) produced no CT by eliminating both inducers of CT expression (*toxR* and *toxT*) directly or indirectly.

Conclusions and Future Studies

We have shown that stringent response regulates both CT and TCP through differential regulation of components of the ToxR regulon. Stringent response is necessary for full production of TCP and colonization of the small intestine.

The differential regulation of *toxRS*, *tcpPH* and *toxT* is interesting and we would like to understand how this regulation occurs and when it occurs during infection.

References

He, H., Cooper, J.N., Mishra, A., and Raskin, D.M. Stringent response regulation of biofilm formation in *Vibrio cholerae*. *J. Bacteriol.* 2012.194:2962-2972.

Zhu, J. and Mekalanos, J.J. Quorum sensing dependent biofilms enhance colonization in *Vibrio cholerae*. *Dev. Cell* 2003. 5:647-656.

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