Regulation of *Vibrio cholerae* virulence in response to novel host signals

Pratik Shah[1,2,3] and Deborah Hung[1,2,3]

[1]Infectious Disease Initiative, Broad Institute of MIT and Harvard;
[2]Department of Molecular Biology, Massachusetts General Hospital;
[3]Department of Microbiology and Immunology, Harvard Medical School

Vibrio cholerae, a Gram-negative bacterium, causes the human diarrheal disease cholera. As a model organism, it is a genetically tractable system for understanding bacterial pathogenesis, as evidenced by the successful identification of some of its virulence factors. Yet, much remains unknown with respect to its' mechanisms to sense and respond to virulence activating stimuli and metabolites within the host microenvironment. Anaerobic growth has been shown to increase virulence gene expression in Gram-negative enteric and non-enteric bacteria. *V. cholerae* is subjected to an oxygen-gradient during colonization of the host intestine leading to disease, suggesting a link between hypoxia and virulence gene expression. A non-redundant and arrayed transposon library was screened to identify two-component system (TCS) mutants showing significant reduction in cholera toxin (CT) production under microaerophilic conditions compared to an isogenic wild-type parent. Four unique TCS that potentially sense and respond to oxygen, alternative electron acceptors or osmolarity were identified. In-frame unmarked deletion strains of the identified TCS sensor and the cognate regulator proteins showed reduction in CT production under hypoxic conditions and were significantly attenuated in an infant mouse model of *V. cholerae* colonization (P less than 0.05). Digital gene expression technology revealed that *V. cholerae* spatio-temporally regulate CT expression during infection. Our data thus provides an association between the host milieu, sensing and signaling mechanisms and temporal regulation of virulence in *V. cholerae* and potentially other intestinal bacterial pathogens of humans.

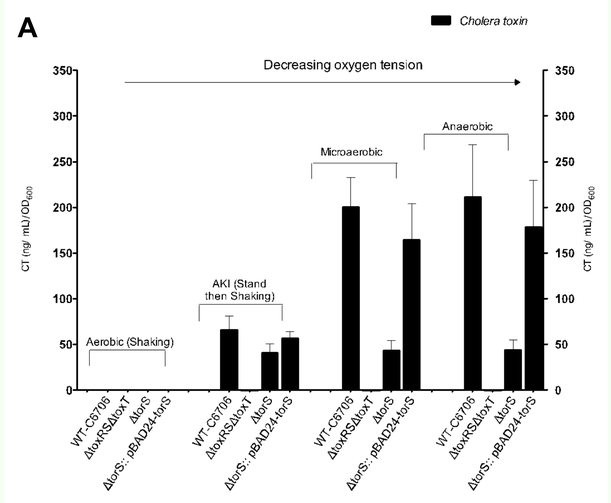
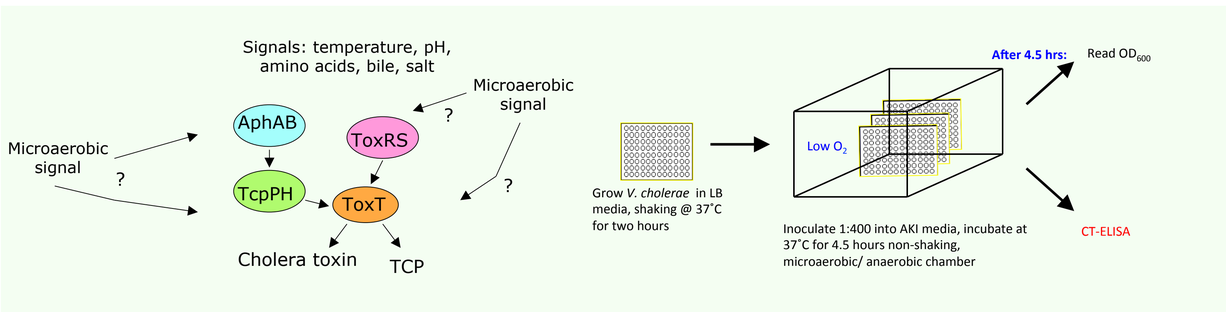


Figure 2A and B. The *tor* phosphorelay system regulates cholera toxin production during microaerobic growth in wild-type *V. cholerae*



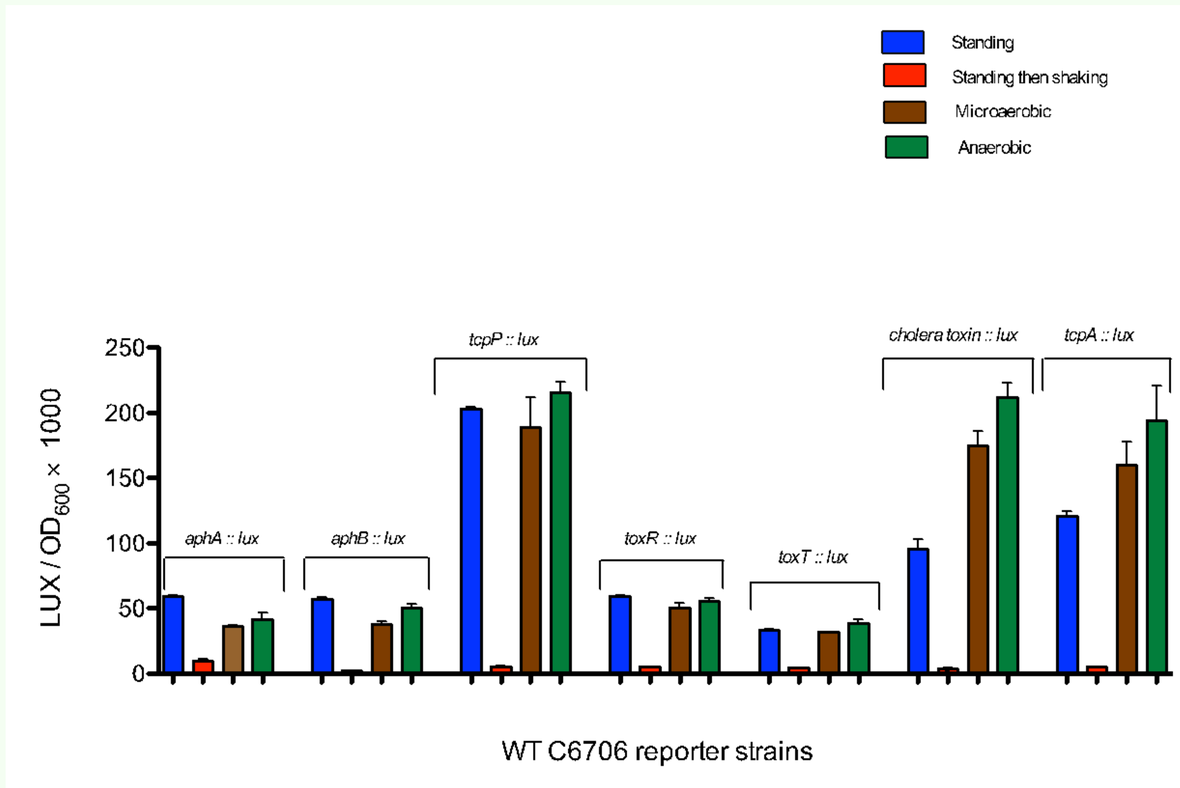


Figure 1. Microaerobic growth induces virulence gene expression in *V. cholerae*

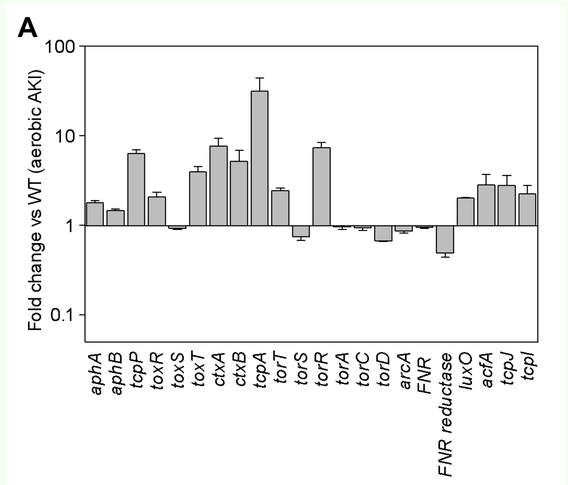


Figure 3A. Expression of *torR* is increased during microaerobic growth

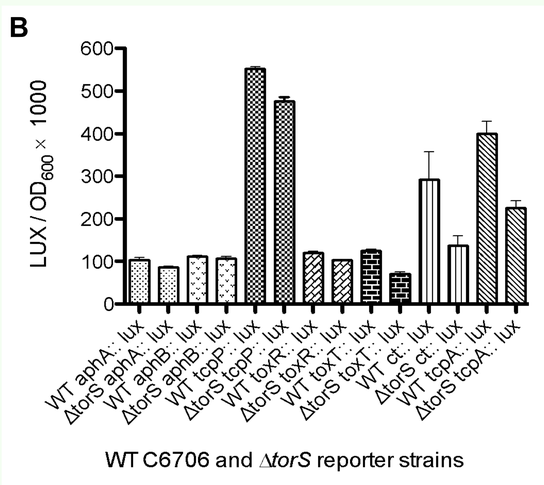


Figure 3B. *torS* regulates virulence expression by reducing *tcpP* transcription

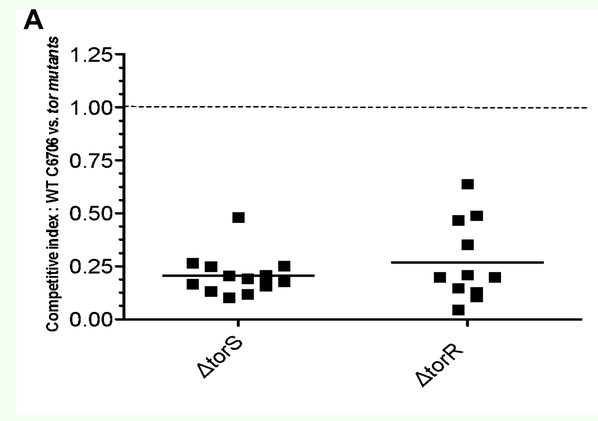


Figure 4A. The *tor* regulon is essential for successful colonization of the infant mouse intestine

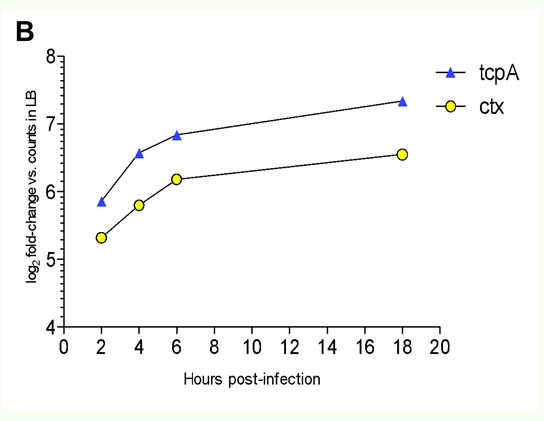


Figure 4B. Digital gene expression analysis indicate temporal regulation of virulence expression during infection