Sequencing is believing: A *V. cholerae* Type Three Secretion System Story

Michelle Dziejman
Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY

Although the toxin co-regulated pilus (TCP) and cholera toxin (CT) are essential virulence factors used by epidemic O1 and O139 serogroup strains of *V. cholerae* to colonize the human host and cause disease, other strains of *V. cholerae* cause cholera-like symptoms using different virulence factors. For example, a subset of pathogenic, non-O1/non-O139 serogroup strains use a Type Three Secretion System (T3SS) to promote disease. Originally identified by genomic sequence analysis of an O39 serogroup strain named AM-19226, the *V. cholerae* T3SS is most similar to the T3SS2 present in pandemic *V. parahaemolyticus* strains. The genes encoding the structural subunits of the AM-19226 T3SS are found on a ~50kb pathogenicity island that also encodes two ToxR-like transcriptional regulators, VttRA and VttRB. Both proteins are critical for virulence *in vivo*, and *in vitro* data suggest that both proteins have a role in bile dependent activation of T3SS genes. T3SS effector proteins are typically not conserved across multiple species; we therefore considered hypothetical proteins (HPs) encoded within the AM-19226 T3SS as potential effector proteins. Using *S. cerevisiae* as a model system, we surveyed more than 30 HPs for their ability to inhibit growth when expressed in yeast, based on the premise that effector proteins can disrupt cell homeostasis and signaling pathways. We then subsequently tested ORFs of interest for T3SS dependent translocation into HeLa cells using a FRET-based assay. Our results have identified several novel effector proteins encoded by the AM-19226 T3SS island, and we hypothesize that their cooperative activities promote cholera in the absence of TCP and CT. Questions about how unique effector proteins interact with host cell components and how T3SS related virulence genes are regulated therefore represent new challenges in understanding the molecular mechanisms underlying TCP/CT independent cholera as a diarrheal disease.