The ZorO Type I Toxin: Controlling a Frienemy

In recent years, a plethora of type I toxin-antitoxin loci have been identified within the chromosomes of a variety of bacterial species. These loci consist of a small, hydrophobic toxic protein and a small RNA (sRNA) antitoxin that represses toxin translation by binding to the toxin mRNA. Previously, we confirmed that the *zorO-orzO* gene pair in the pathogen *Escherichia coli* O157:H7 is a bona fide toxin-antitoxin locus, with the *zorO* gene encoding the toxin, and *orzO*, the antitoxin.

We noted that OrzO base pairs to the 5' untranslated region (UTR) of the *zorO* mRNA, approximately 60 nucleotides upstream of the ribosome binding site for the toxin. Thus, repression by OrzO does not occur by binding over the translation initiation region of *zorO*. How then can the antitoxin repress expression of the toxin? We hypothesized that there are sequence and structural elements within the *zorO* 5' UTR that are required for its translation and that binding of OrzO disrupts these features. Truncations of the 5' UTR had significant impacts on ZorO toxicity to *E. coli*, reflective of the importance of post-transcriptional regulation controlling toxin production. Additionally, we could transfer these effects on protein production by generating fusions of the *zorO* UTR onto the reporter gene *gfp*, confirming the importance of this untranslated region in gene expression.

Along with regulatory control, we have begun studies to elucidate how the ZorO toxin functions in *E. coli.* Predictive tools indicate that ZorO is membrane localized and could possibly dimerize forming a pore. Indeed, overproduction of ZorO leads to dissipation of proton motive force. The ZorO protein is unusual in that it has numerous charge residues thought to reside within the membrane domain; our preliminary data suggests these residues are critical for its toxicity. Further studies are ongoing to examine how these impact the function of the protein.