

Targeted Regulation of Protein Expression in *Vibrio parahaemolyticus*

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Abstract

V. parahaemolyticus has several virulence factors, including thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH), and two separate type III secretion systems (T3SSs), T3SS1 and T3SS2. T3SS1 is responsible for cytotoxicity, primarily through the activity of its effector VP1680. To gain a detailed understanding of the relationship between the amount of effector, its expression timing, and cytotoxicity, a system is required to regulate protein expression levels and timing. In the present study, we developed an effector protein expression system controlled by an arabinose-dependent transcription factor and found that cytotoxicity toward mammalian cells increased in a VP1680-dependent manner. To ensure specific protein degradation, we also established a targeted protein degradation system, including VP0917 (ClpP) and VP0918 (ClpX)-, or VP0917 and VP1014 (ClpA)-mediated degradation of *ssrA*-tagged proteins (proteins bearing the C-terminal degradation tag encoded by *tmRNA*). By combining these systems, more than 50% of the targeted protein could be degraded within 20 min. As a byproduct of creating the systems, we obtained an enhanced green fluorescent protein variant that emits strong fluorescence in *V. parahaemolyticus*. The protein degradation system developed in this study has demonstrated the potential to control intracellular protein levels to a certain extent. Moreover, experimentally controlling intracellular protein levels will allow for a more detailed examination of the relationship between protein quantity and cellular phenotype, potentially overcoming the limitations of the “all-or-nothing” model.

Keywords:

V. parahaemolyticus; pBAD/AraC; trans-translation; *ssrA*