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Regulatory Cascade for Quorum-Sensing Mechanism in *Vibrio vulnificus*

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Vibrio vulnificus, a human pathogen causing fatal septicemia, shows rapid pathogenic progresses and high mortality rates. One of the major virulence factors responsible for this pathology is an extracellular protease called elastase or VvpE [1, 2]. It possesses the ability to enhance vascular permeability, to cause hemorrhagic damage, and to degrade type IV collagen in the vascular basement membrane (BM), which may cause destruction of the BM and breakdown of capillary vessels. Expression of *vvpE* was found to be induced under the conditions at high cell density, and its regulation was shown to be mediated by sigma factor S, cAMP-CRP, and SmcR [3, 4].

SmcR, one of the regulators of *vvpE* expression, is homologous to *V. harveyi* LuxR, which is a master quorum-sensing regulator. In related pathogens, *V. cholerae*, *V. anguillarum*, and *V. parahaemolyticus*, their virulence factors, such as hemagglutinin/protease, EmpA (metalloprotease), and capsular polysaccharide are regulated by LuxR-homologous regulators, HapR, VanT, and OpaR, respectively. Thus, fine-tuning of expression of genes controlled by quorum sensing is achieved by modulation of intracellular levels of this transcriptional factor, LuxR. LuxR synthesis has been reported to be regulated at the post-transcriptional level in *V. harveyi* [5]. Under the low concentration of quorum sensing signals, the phosphorylated form of LuxO represses *luxR* expression by activating the transcription of five sRNAs. The sRNAs in the presence of Hfq destabilize the *luxR* mRNA, and thus repress LuxR synthesis. The same mechanism was also operative in repression of *hapR* expression by four sRNAs in *V. cholerae*. There has been no report on the transcriptional control of *luxR*-homologous genes via quorum sensing regulatory cascade, yet.

In this study, transposon mutagenesis of *V. vulnificus* aimed at searching for other regulators of expression of extracellular proteases revealed that *vvpE* expression was increased in a *luxO* mutant. The *luxAB*-transcriptional fusions containing different lengths of the *smcR* promoter region indicated that the *smcR* transcription was negatively regulated by LuxO, and the specific upstream region of the *smcR* gene

was required for this repression. Since LuxO is a known member of a positive regulator, the negative regulation of *smcR* transcription by LuxO stimulated us to identify a factor linking LuxO and the *smcR* transcription. The LuxT protein was isolated in a ligand-fishing experiment using the *smcR* promoter as bait, and the *smcR* expression was shown to be increased by *luxT* mutation. The recombinant LuxT protein bound to a specific region of the *smcR* promoter. The expression of *luxT* was positively regulated by LuxO, and the promoter of *luxT* contained a putative LuxO-binding site. Mutagenesis of the LuxO-binding site in the *luxT* promoter region resulted in a loss of transcriptional control by LuxO. Therefore, this study demonstrates LuxT as a transcriptional factor regulating *smcR* expression, which relays information of quorum signal sensed by LuxO to production of an extracellular protease, VvpE.

References

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