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Molecular Characterization of Survival and Toxigenesis of *Vibrio vulnificus*

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ABSTRACT

Understanding the molecular pathogenesis of the multifaceted host-pathogen interaction is critical in the development of improved treatment and prevention, as well as elucidating how certain bacteria can circumvent host defenses, multiply in the host, and cause such extensive damage. Disease caused by infection with *V. vulnificus* is remarkable for the invasive nature of the infection, ensuing severe tissue damage, and rapidly fulminating course. The characterization of somatic as well as secreted products of *V. vulnificus* has yielded a large list of putative virulence attributes, whose known functions are easily imagined to explain the pathology of disease. These putative virulence factors include a carbohydrate capsule, lipopolysaccharide, a cytolysin/hemolysin, elastolytic metalloprotease, iron sequestering systems, lipase, and pili. However, only few among the putative virulence factors has been confirmed to be essential for virulence by the use of molecular Koch's postulates. This presentation describes molecular biological characterization of the virulence factors contributing to survival as well as to toxigenesis of *V. vulnificus*.

1. Modulation of Acid-Induced Expression of the *Vibrio vulnificus cadBA* genes by CadC

Like many other food-borne pathogenic bacteria, *V. vulnificus* has to cope with ever-changing acidity in their growth environments to ensure developing illness. The *V. vulnifcius cadBA* genes encode a lysine/cadaverine antiporter and a lysine decarboxylase, whose combined activity leads to the synthesis and excretion of cadaverine to counteract external acidification. Lysine decarboxylase activity of *V. vulnificus* was induced at a low pH (pH 5.8), and the induction of lysine decarboxylase was regulated at the level of transcription. A primer extension analysis revealed that *cadBA* genes are organized as a single transcriptional unit, and that the transcription of *cadBA* begins at a specific site, consisting of a putative promoter P_{cad}. An open reading frame, *cadC*, consisting of 526 amino acids, was identified upstream of P_{cad}. The production of lysine decarboxylase and the cellular level of *cadBA* transcript decreased in *cadC* mutant, which was constructed by allelic exchange. This decrease in the level of *cadBA* transcript in the *cadC* mutant appeared to be mediated by the reduced activity of P_{cad}. These results establish that *cadBA* expression is directed by P_{cad} in a pH-dependent manner, and activated by the gene product of *cadC*.

2. Structure and Regulation of the *putAP* Operon Encoding Proline Dehydrogenase and Proline Permease of *Vibrio vulnificus*

The *Vibrio vulnificus* putAP genes encode proline dehydrogenase and proline permease involved in proline catabolism and proline uptake, respectively. The gene product of *putP* also contributes to the osmotic tolerance of *V. vulnificus*. In this study, we demonstrated that proline dehydrogenase activity of *V. vulnificus* was highly dependent on growth phase, reached a maximum during the exponential phase and then decreased when cells enter the stationary phase. This growth phase variation of proline dehydrogenase occurred at the level of transcription. Northern blot and primer extension analyses revealed that *putA* and *putP* are organized as one transcriptional unit, and the transcription of *putAP* operon begins at a site, consisting of a putative promoter P_{put}. A null mutation of *crp* decreased proline dehydrogenase production and the cellular level of *put* transcript, indicating transcription of *put* is under the positive control of CRP. This decrease in the level of *put* transcript in the *crp* mutant appeared to be mediated by the reduced activity of P_{put}. Proline dehydrogenase was induced in the presence of proline, and the induction of proline dehydrogenase was regulated at the level of transcription. A transcriptional attenuation was observed after the *putA* gene, so *putP* expression was reduced presumably to modulate the production of the proline permease according to the available concentration of proline. These results establish that *putAP* expression is directed by P_{put} in a growth phase-dependent manner, and induced by the gene product of *crp* in the presence of proline.

3. Promoter Analysis and Regulatory Characteristics of *vvhBA* Encoding Cytolytic Hemolysin of *Vibrio vulnificus*

Hemolysin, a gene product of *vvhA*, is a potent virulence factor of the pathogenic bacterium *V. vulnificus*, and shows cytolytic activity. Hemolysin activity and the level of *vvh* transcript reached a maximum in the late exponential phase and then decreased when cells entered the stationary phase. Northern blot and primer extension analysis revealed that *vvhB* and *vvhA* are organized as one transcriptional unit, and that the transcription of the *vvhBA* operon begins at one site, consisting of a promoter P_{vvh}. A *crp* null mutation decreased hemolysin production and the cellular level of *vvhBA* transcript by reducing the activity of P_{vvh}, indicating that the P_{vvh} activity is under the positive control of CRP. A direct interaction between CRP and the regulatory region of the *vvhBA* operon was demonstrated by a gel-mobility shift assay. The CRP binding site mapped by deletion analysis of the *vvhBA* promoter region and confirmed by DNase I protection assay was centered at the 59.5 base pairs upstream of the transcription start site. These results demonstrate that the *vvhBA* expression is activated by CRP in a growth-dependent manner, and CRP exerts its effects by directly binding to P_{vvh}.

4. Molecular Analysis of the Regulatory Effect of SmcR and CRP on the Expression of Elastase in *Vibrio vulnificus*

Among the putative virulence factors of *Vibrio vulnificus* is an elastase(Hase et al.), the gene product of *vvpE*. It has been demonstrated that *vvpE* expression is differentially directed by two different types of promoters, PL and PS, and elevated by RpoS, CRP and a trans-acting regulatory protein SmcR. In this study, a primer extension analysis revealed that the activity of log-phase promoter, PL, was unaffected by a null

mutation of *smcR*. The mutation of *smcR* reduced the activity of stationary-phase promoter, PS, indicating that the effect of SmcR on the expression of elastase is occurred through PS requiring RpoS. The mutation of *crp* also reduced, even less extent, the PS activity; however, the additional inactivation of CRP did not influence the PS activity in the *smcR* mutant. This indicated that the effect of CRP on the PS activity is mediated by SmcR. GST-pull down and gel-mobility shift analyses revealed that CRP interacted directly with SmcR, and facilitated binding of SmcR to the promoter. The binding sites for CRP and SmcR were mapped by deletion analysis of the *vvpE* promoter region and confirmed by an *in vitro* DNase I protection assay. Each binding site for CRP and SmcR was juxtapositioned and centered at the 214 and 184 base pairs upstream of the transcription start site of PS, respectively. The combined results demonstrate that the activation of *vvpE* expression by by CRP and SmcR is occurred in a growth-dependent manner through PS, and the regulatory proteins exerts their effects by directly binding to the promoter in the stationary
phase.

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