Regulatory roles of sRNAs in pathogenesis of *Vibrio cholerae*

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofi/medicine doktorsexamen framläggs till offentligt försvar i hörsal E 04 Unod R1, Norrlands universitetssjukhus, Fredagen den 27 March 2015, kl. 9:00.

Avhandlingen kommer att försvaras på engelska.

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Abstract
The Gram-negative pathogen *Vibrio cholerae* uses variety of regulatory molecules to modulate expression of virulence factors. One important regulatory element of microorganisms is small non-coding RNAs (sRNAs), which control various cell functions such as expression of cell membrane proteins, mRNA decay and riboswitches. In this thesis studies, we demonstrated the roles of the sRNAs VrrA in regulation of outer membrane protein expression, biofilm formation and expression of ribosome binding proteins. In addition, we showed that VrrB, a newly discovered sRNA, played a role in amino acid dependent starvation survival of *V. cholerae* and might functioned as a riboswitch.

VrrA, a 140-nt sRNAs in *V. cholerae*, was controlled by the alternative sigma factor σ^E_. The outer membrane protein, OmpT is known to be regulated by environmental signals such as pH and temperature via the ToxR regulon and carbon source signals via the cAMP–CRP complex. Our studies provide new insight into the regulation of OmpT by signals received via the σ^E regulon through VrrA. We demonstrated that VrrA down-regulate *ompT* translation by base-pairing with the 5′ region of the *ompT* mRNA in a Hfq (RNA chaperone protein) dependent manner.

*V. cholerae* biofilms contain three matrix proteins—RbmA, RbmC and Bap1—and exopolysaccharide. While much is known about exopolysaccharide regulation, little is known about the mechanisms by which the matrix protein components of biofilms are regulated. In our studies, we demonstrated that VrrA negatively regulated *rbmC* translation by pairing to the 5' untranslated region of the *rbmC* transcript and that this regulation was not stringently dependent on Hfq.

In *V. cholerae*, VC0706 (Vrp) and VC2530 proteins are homologous to ribosome-associated inhibitor A (RaiA) and hibernation promoting factor (HPF) of *Escherichia coli*, respectively. HPF facilitates stationary phase survival through ribosome hibernation. We showed that VrrA repressed Vrp protein expression by base-pairing to the 5′ region of *vrp* mRNA and that this regulation required Hfq. We also showed that Vrp was highly expressed during stationary phase growth and associated with the ribosomes of *V. cholerae*. We further demonstrated that Vrp and VC2530 were important for *V. cholerae* starvation survival under nutrient-deficient conditions. While VC2530 was down-regulated in bacterial cells lacking vrrA, mutation of *vrp* resulted in increased expression of VC2530.

Riboswitches are an important class of regulators in bacteria, which are most often located in the 5′ untranslated region (5′ UTR) of bacterial mRNA. In this study, we discovered the novel non-coding sRNA, VrrB located at the 5′ UTR of a downstream gene encoding Vibrio auxotrophic factor A (VafA) for phenylalanine. In *V. cholerae*, reduced production of VafA was observed in the presence of phenylalanine and phenylpyruvate in the culture media. Some analogs of phenylalanine and phenylpyruvate could also modulate the expression of VafA. Furthermore, bacterial cells lacking the vrrB gene exhibited high production of VafA, suggesting that VrrB might function as a riboswitch that controls VafA expression.