Analysis of the *vlpA* genes and their roles in the pathogenesis of *Vibrio cholerae* O139.

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Abstract

The *vlpA* (*Vibrio cholerae* lipoprotein A) gene encodes the first identified bacterial member of the α_2-microglobulin (lipocalin) superfamily, which function as small hydrophobic molecule transporters. This gene is present in *V. anguillarum*, *V. cholerae* O1, and some strains of non-O1 serotypes in multiple copies and flanked by a repeat sequence of approximately 124-bp, termed VCR for *V. cholerae* repeat sequence. The VCR element has all the structural features associated with the 59-base element (59-be) (or *attC* site) required for site-specific recombination mediated by integron encoded integrases. Over 150 copies of this VCR element are present in the *V. cholerae* genome, interspersed with open reading frames or gene cassettes. This constitutes the *V. cholerae* mega-integron, a chromosomally located integron of approximately 120-kb. Both integrases IntI1 from *Pseudomanas aeruginosa* and IntI4 from *V. cholerae* are capable of mediating deletion of gene cassettes containing VCRs in plasmid clones in *Escherichia coli*, and in *V. cholerae* chromosomally located *vlpA* genes. The deletion event occurs at the VCR sites in the presence of an over-expressed clone of both IntI1 and IntI4. Interestingly, IntI4 specificity is not limited to the VCR elements of *V. cholerae*, but also recognizes the *attI* site of the *P. aeruginosa* integron. However, the deletions require the presence of the *attI*1 site in addition to the VCR elements for IntI1 mediated excision. This is also the case for IntI4 which requires the *attI*4 site as well as VCRs for activity. Site-specific recombination catalyzed by IntI1 occurs at both VCR and *attI*1 sites, whereas IntI4 was shown to capable of mediating deletion only at VCR sites. This is the first time IntI4 has been shown to function as an integrase mediated the deletion of gene cassettes in the *V. cholerae* chromosome.

A mutant lacking VlpA activity has been constructed in *V. cholerae* O139 which contains two copies of *vlpA*, by allelic exchange after inactivation via the insertion of kanamycin and tetracycline resistance gene cartridges. The VlpA-negative mutant has been assessed for virulence in the infant mouse cholera model. This mutant does not show a marked defect in its ability to persist in the infant mouse gut and competes equally well with the wild-type organism. The function of VlpA is still unknown, although the eukaryotic lipocalins are best known for their binding of small hydrophobic ligands.

Different promoters from plasmid vectors were used in *E. coli* and *V. cholerae* to express *vlpA*, but a gene product was detected in *E. coli* using the T7 phage expression system, with a protein size of ~19 kDa. No specific products with Western blotting analysis were present to show that VlpA was expressed in *V. cholerae*, even in iron limiting conditions. Northern blot analysis, primer extension, and RT-PCR were utilized to determine if transcription of *vlpA* occurred, but its expression from the *V. cholerae* chromosome is still unclear.

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