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Molecular characterization of the BvgA response regulator of *Bordetella holmesii*

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Summary

The BvgAS system controls the expression of most virulence factors in *Bordetella pertussis*. Recently, we identified an orthologous system in the related human pathogen *Bordetella holmesii*. However, while we found that the orthologous histidine kinases BvgS could be functionally exchanged between the two species, the *B. holmesii* response regulator $BvgA_{BH}$ could not substitute for its *B. pertussis* counterpart *in vivo* and, accordingly, was not able to bind to *B. pertussis* virulence promoters *in vitro*. Here we show that a hybrid response regulator consisting of the *B. pertussis* derived DNA-binding output domain of $BvgA_{BP}$ combined with the *B. holmesii* receiver domain binds to $BvgA_{BP}$ regulated virulence promoters of *B. pertussis in vitro* and is functional in *B. pertussis in vivo*. This shows that the inability of $BvgA_{BH}$ to complement $BvgA_{BP}$ in *B. pertussis* is due to the small number of sequence variations present in its output domain. However, by mutation analysis we show that four amino acid exchanges present in the helix-turn-helix motif of $BvgA_{BH}$ as compared to $BvgA_{BP}$ are not the only reason for its inability to substitute for $BvgA_{BP}$ but additional mutations present in the output domain must play a role.

Introduction

The BvgAS two-component system is the master regulator of virulence gene expression in the human and mammalian pathogens *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* (Bock and Gross, 2001; Cotter and Jones, 2003). *B. pertussis* is the causative agent of whooping cough in man, *B. parapertussis* causes milder forms of the disease, and *B. bronchiseptica* causes respiratory infections in many mammals (Mattoo and Cherry, 2005). Among the virulence genes positively controlled by the BvgAS system there are adhesins such as the filamentous haemagglutinin (FhaB) (Boucher et al., 2003) and

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toxins such as the adenylate cyclase toxin (CYA) which has haemolytic activity (Steffen et al., 1996). Moreover, the *bvgAS* locus is positively autoregulated (Scarlato et al., 1991). Signal transduction by the BvgAS system involves a phosphorelay between conserved histidine and aspartate residues located in the unorthodox histidine kinase BvgS and the response regulator BvgA (Uhl and Miller, 1994;Perraud et al., 1998). Since recently, "new" *Bordetella* species were increasingly recognized to be associated with disease in man (Gerlach et al., 2001; Mattoo and Cherry, 2005). Among these new species *Bordetella holmesii* is of particular interest because it is associated with pertussis-like symptoms in infected patients (Mazengia et al., 2000;Mattoo and Cherry, 2005). Its phylogenetic position in the genus *Bordetella* is not clear, since its 16S rDNA sequence places this organism close to *B. pertussis* while other phylogenetic parameters indicate this pathogen to be more closely related to animal pathogens such as *Bordetella avium* (von Wintzingerode et al., 2002). Recently, we were able to identify a two-component system of *B. holmesii*, which very much resembles the BvgAS two-component systems of *B. pertussis* and *B. bronchiseptica* (Gerlach et al., 2004) and also regulates putative virulence genes orthologous to those of *B. pertussis* in *B. holmesii* (Link, et al., unpublished results). An initial characterization of this two-component system revealed that the orthologous *bvgS* genes encoding the histidine kinases can be functionally exchanged between *B. pertussis* and *B. holmesii*. In contrast, the BvgA protein ($BvgA_{BH}$) of *B. holmesii* was found not to be functional in *B. pertussis*, despite the fact that the two orthologous proteins are highly similar and that $BvgA_{BH}$ could be phosphorylated by the heterologous histidine kinase. In line with this finding, *in vitro* DNA binding experiments with target promoters of the BvgA protein of *B. pertussis* ($BvgA_{BP}$) including the *fhaB* promoter were not successful with the $BvgA_{BH}$ protein of *B. holmesii*.

Two-component response regulators have a modular architecture with two independent domains, the phosphate-accepting receiver and the DNA binding output domain which are connected by a flexible linker region (Weiss and Stock, 2001). Recently we defined the domain borders and the linker sequence of the *B. pertussis* $BvgA_{BP}$ protein by an approach combining limited proteolysis and mass spectrometry (Bock et al., 2001). Based on this information we constructed a response regulator hybrid between the BvgA proteins of *B. pertussis* and *B. holmesii* and analysed its DNA-binding and virulence gene activating properties in comparison with those of the $BvgA_{BP}$ and $BvgA_{BH}$ proteins in order to understand the functional differences between these highly related proteins.

Materials and methods

Bacterial strains and growth conditions

Bacterial strains used in this study are listed in Table 1. *Bordetella* strains were grown in modified Stainer–Scholte (SS) medium (Stainer and Scholte, 1979; Aoyama et al., 1986) or on Bordet–Gengou (BG) agar plates supplemented with 20% horse blood (Bordet and Gengou, 1909). *Escherichia coli* strains were cultivated in Luria–Bertani (LB) broth. If required, antibiotics were added in the following final concentrations: streptomycin, 100 $\mu g\,ml^{-1}$; kanamycin, 25 $\mu g\,ml^{-1}$; ampicillin, 100 $\mu g\,ml^{-1}$; and chloramphenicol, 20 $\mu g\,ml^{-1}$.

General techniques

DNA manipulations, cloning procedures and SDS-PAGE were carried out according to standard procedures (Sambrook and Russel, 2000). PCR amplifications were performed with a model Progene thermocycler (Techne) by using Pfu DNA polymerase (Promega) or Taq polymerase (MP Biomedicals). All PCR products were subjected to automated sequencing (Big Dye kit; Perkin-Elmer). Mutagenesis was performed with the Quik-Change™ site-directed mutagenesis kit (Stratagene) as described by the supplier. Conjugational DNA transfer into *B. pertussis* was carried out as described previously (Gross and Rappuoli, 1988) with *E. coli* strain SM10 as a donor (Simon et al.,1983). Oligonucleotides used in this study are listed in Table 2.

Construction of the hybrid response regulator $BvgA_{fus}$

In order to assemble the plasmid pSK-$bvgA_{fus}$ the 426 bp DNA region encoding the receiver and linker domains of $BvgA_{BH}$ of *B. holmesii* was amplified by PCR using the primer pair $bvgA_{BH}$BamHIfor and $bvgA_{BH}$EcoRIrev from chromosomal DNA of the *B. holmesii* strain G7702. The 197 bp DNA segment containing the output domain of $BvgA_{BP}$ of *B. pertussis* was amplified by PCR with the primers $bvgA_{BP}$-*O*-EcoRI and $bvgA_{BP}$-*O*-KpnI from chromosomal DNA of *B. pertussis* strain TI. The PCR fragments were digested with *Eco*RI and ligated. Then the primers $bvgA_{BH}$BamHIfor and