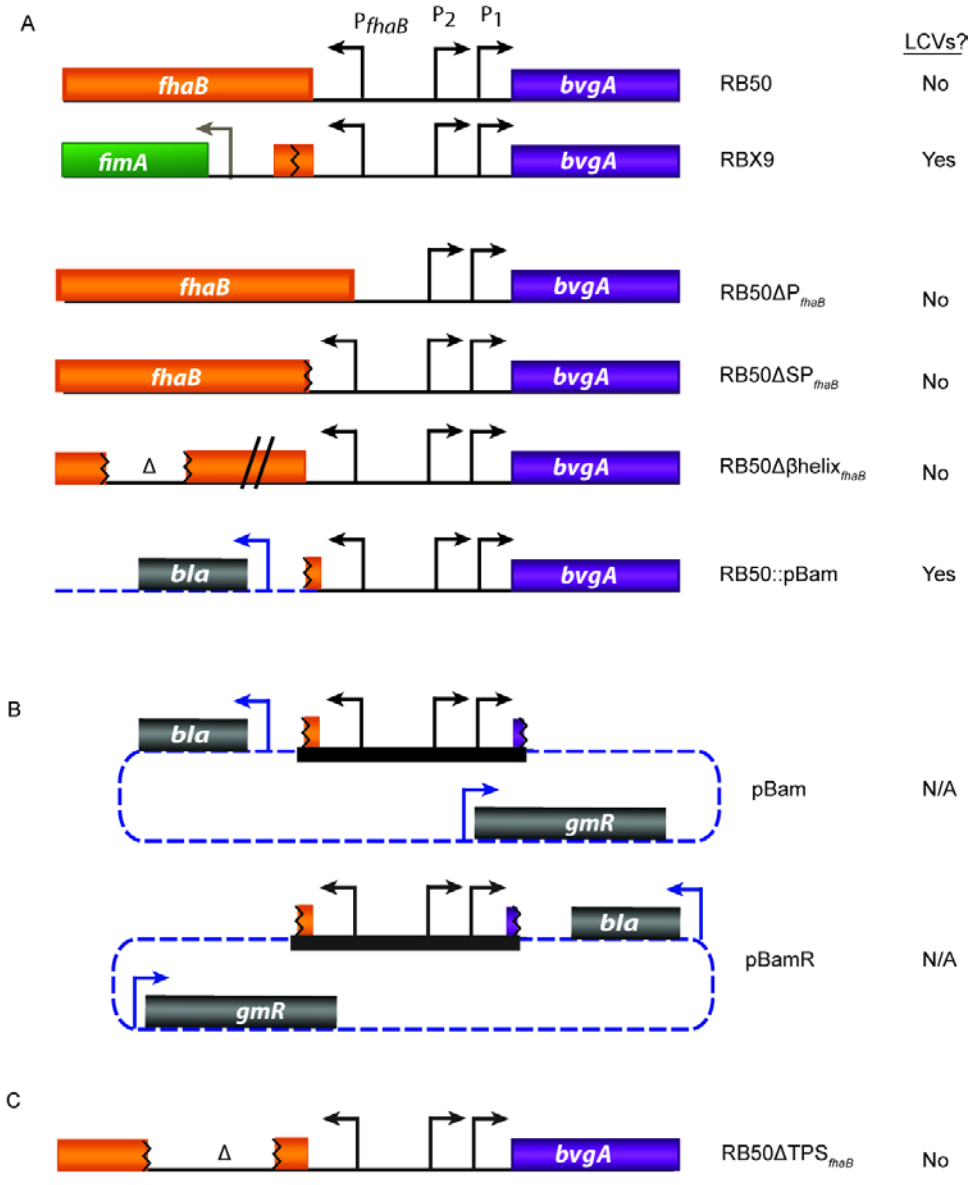
Evidence for phenotypic bistability resulting from transcriptional interference of *bvgAS* in *Bordetella bronchiseptica*

Eliza Mason[1], Michael W. Henderson[1], Erich V. Scheller[1], Matthew S. Byrd[1], Peggy A. Cotter[1]*

[1]Department of Microbiology and Immunology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599-7290, USA.
*For correspondence. E-mail peggy_cotter@med.unc.edu; Tel. (+1) 919 966 5612; Fax (+1) 919 962 8103

Supporting Information

Supplemental Figure 1



Supplemental Figure 1. A, genetic architecture of strains that do and do not produce LCVs, including RB50ΔP_{fhaB}, RB50ΔSP_{fhaB}, RB50Δβhelix$_{fhaB}$, and RB50::pBam with RB50 and RBX9 as a reference; B, schematic of pBam and pBamR plasmids and the orientation of their inserted sequences; blue dotted lines represent plasmid DNA; thick black lines represents *fhaB-bvgAS* intergenic region; C, Genetic architecture of strain RB50ΔTPS_{fhaB}; not drawn to scale.

Supplemental Table 1: Strains and Plasmids used in this study

Strain or Plasmid	Description	Reference
Strains		
E. coli		
DH5α	Molecular cloning strain	(Inatsuka *et al.*, 2010)
RH03	Conjugation strain; Km^s, DAP auxotroph	(López *et al.*, 2009)
Bordetella		
RB50	Wild-type *B. bronchiseptica* strain; Sm^r	(Cotter and Miller, 1994)
RBX9	RB50 with an in-frame deletion mutation of *fhaB*	(Cotter *et al.*, 1998)
RB53	RB50 with the *bvgS*-C3 mutation (Bvg^+ phase-locked)	(Cotter and Miller, 1994)
RB50i	RB50 with the *bvgS*-I1 mutation (Bvg^i phase-locked)	(Cotter and Miller, 1997)
RBX9i	RBX9 with the *bvgS*-I1 mutation (Bvg^i phase-locked)	(Cotter and Miller, 1997)
RBX9c	RBX9 with *bvgS*-CS3 mutation (Bvg^+ phase-locked)	This study
RB50::pBam	RB50 with pBam integrated between *bvgA* and *fhaB*; Gm^r	This study
RB50::pBamR	RB50 with pBamR integrated between *bvgA* and *fhaB*; Gm^r	This study
RB50Δ*fhaB*	RB50 with same Δ*fhaB* mutation as RBX9	This study
RBX9BatBN-HA*flaA-gfp*	RBX9 with N-terminal HA-encoding tag in *batB* (proceeding codon 55) and P_{flaA} *gfp* at *att*Tn7	This study
RB50ΔP_{fhaB}	RB50 with a deletion mutation of the *fhaB* promoter (nt -244 through -28 relative to the ATG)	This study
RB50ΔSP_{fhaB}	RB50 with a *fhaB* signal peptide deletion mutation (codons 2-70)	This study
RB50Δβhelix$_{fhaB}$	RB50 with an in-frame deletion mutation of codons 385 -1979 of *fhaB* (encoding the β-helix)	This study
RB50ΔTPS_{fhaB}	RB50 with a deletion mutation of bp 8 - 1256 (within codons 3 - 413) in *fhaB*	This study
RBX9*cyaA*FLP	RBX9 with *flp* driven by P_{cyaA} integrated at *att*Tn7	This study
RB50P_{short}*bvgAFLP*	RB50 with *flp* driven by $P_{bvgA\text{-}short}$ integrated at *att*Tn7	This study
RB50P_{long}*bvgAFLP*	RB50 with *flp* driven by $P_{bvgA\text{-}long}$ integrated at *att*Tn7	This study
RBX9F	RBX9 with a deletion mutation of the *fimA-fhaB* intergenic region	This study

RBX9cF	RBX9ΔP*fimA* with the *bvgS*-CS3 mutation	This study
Plasmids		
pSS4245	pBR322-based allelic exchange plasmid; Ap^r, Km^r	(Inatsuka *et al.*, 2010)
pEG7S	*Bordetella* allelic exchange vector; Ap^r, Gm^r	(Julio and Cotter, 2005)
pEG7	pBR322-based suicide plasmid; Ap^r, Gm^r	(Cotter and Miller, 1997)
pEG3SO	Suicide plasmid encoding *bvgS*-CS3 mutation (R570H) with flanking sequence	(Cotter and Miller, 1994)
pΔP*fhaB*	pEG7S derivative with nt (-29) – (500) and (-750) – (-243) relative to *fhaB* ATG	This study
pΔSP*fhaB*	pEG7S derivative with sequences comprising codons 30 of *bvgA* to codon 1 of *fhaB* and codons 71-238 of *fhaB*	This study
pΔβhelix*fhaB*	pSS4245 derivative with codons 218-235 and 1979-2146 of *fhaB*	This study
pΔTPS*fhaB*	pSS4245 derivative with sequences from codon 3 of *fhab* through codon 30 of *bvgA* and codons 413-567 of *fhaB*	This study
pX9ΔP*fimA*	pSS4245 derivative with codons 1-183 of *fimA* and 16 bp 3' to the *fhaB* STOP codon through codon 30 of *bvgA* of RBX9	This study
pGFLIP	Tn7-based vector with PS12-*gfp* and nptII flanked by FRT sequences and *flp* 3' to the MCS; Ap^r, Km^r (conditional)	(Byrd *et al.*, 2013)
pGFLIP-P*cyaA*	pGFLIP with *flp* driven by the RB50 *cyaA* promoter, Ap^r, Km^r (conditional)	(Byrd *et al.*, 2013)
pGFLIP-P*flaA*	pGFLIP with *flp* driven by the RB50 *flaA* promoter, Ap^r, Km^r (conditional)	(Byrd *et al.*, 2013)
pGFLIP-P*bvgA-short*	pGFLIP with *flp* driven by the *fhaB-bvgAS* intergenic region	This study
pGFLIP-P*bvgA-long*	pGFLIP with *flp* driven by sequences 1200bp of *fhaB* to the *bvgAS* translational start site	This study
pTnS3	Tn7 transposase expression vector containing *tnsABCD*; Ap^r	(Choi *et al.*, 2008)
pBam	pEG7 plasmid with *bvgAS-fhaB* intergenic region in the MCS	This study
pBamR	pEG7 plasmid with *bvgAS-fhaB* intergenic region (reverse orientation relative to pBam)	This study
pUC18Tn7-*flaA-gfp*	Tn7-based vector with P*flaA*-*gfp;* Ap^r, Km^r	This study
pUC18T-mini-Tn7T-Km-*FRT*	Mobilizable transposition vector; Ap^r, Km^r	(Choi *et al.*, 2008)
pCW103	pSS4245 derivative with HA-encoding sequence flanked by *batB* sequences nt (-300 from ATG) to codon 55 and codons 56-216.	This study

Strain Construction

Allelic exchange was done using derivatives of pEG7S or pSS4245 according to (Tejada *et al.*, 1996; Inatsuka *et al.*, 2010). All strains were confirmed by PCR and nucleotide sequence analysis.

RB50Δ*fhaB* was created by performing allelic exchange on RB50 using plasmid pΔ*fhaB*new.

RBX9BatBN-HA*flaA-gfp* was created by first performing allelic exchange on RBX9 using plasmid pCW103. To the resulting strain, the miniTn7-*flaAgfp* construct was delivered via tronsposase-mediated insertion.

RB50ΔP_{fhaB} was created by performing allelic exchange on RB50 using plasmid pΔP_{fhaB}.

RB50ΔSP_{fhaB} was created by performing allelic exchange on RB50 using plasmid pΔSP_{fhaB}.

RB50Δβhelix$_{fhaB}$ was created by performing allelic exchange on RB50 using plasmid pΔβhelix$_{fhaB}$.

RB50::pBam was created by introducing plasmid pBam into RB50 by conjugation and selecting co-integrants on BG Sm Gm agar as described by Akerley et al. 1995.

RB50::pBamR was created by introducing plasmid pBamR into RB50 by conjugation as described for RB50::pBam.

RBX9*cyaAFLP* was created by delivering pGFLIP-P_{cyaA} construct into the RBX9 chromosome via transposase-mediated insertion and selecting on BG Sm Km + 50mM $MgSO^4$.

RBX9*flaAFLP* was created by delivering pGFLIP-P_{flaA} construct into the RBX9 chromosome via transposase-mediated insertion and selecting on BG Sm Km.

RB50P_{short}*bvgAFLP* was created by delivering pGFLIP-$P_{bvgA\text{-}short}$ construct into the RB50 chromosome via transposase-mediated insertion and selecting on BG Sm Km + 50mM $MgSO^4$.

RB50P_{long}*bvgAFLP* was created by delivering pGFLIP-$P_{bvgA\text{-}long}$ construct into the RB50 chromosome via transposase-mediated insertion and selecting on BG Sm Km + 50mM $MgSO^4$.

RB50ΔTPS was created by performing allelic exchange on RB50 using plasmid pΔTPS_{fhaB}.

RBX9F was created by performing allelic exchange on RB50 using plasmid pΔP_{fimA}.

RBX9c was created by performing allelic exchange on RBX9 using plasmid pEG3S0 according to Cotter et al 1994.

RBX9cF was created by performing allelic exchange on RBX9F using plasmid pEG3SO.

Plasmid Construction

All plasmids were confirmed by PCR and nucleotide sequence analysis.

p∆*fhaB*new was created by PCR amplifying the 1kb region flanking the in-frame deletion of *fhaB* of RBX9 and ligating this insert into the MCS of pSS4245.

p∆P_{fhaB} was created by PCR amplifying one fragment (corresponding to nt -574 through -244 relative to the *fhaB* ATG) ligated to another fragment (corresponding to nt -28 through 470) of RB50 and ligating this ~1kb insert into the MCS of pSS4245.

p∆SP_{fhaB} was created by PCR amplifying one 500bp fragment (corresponding to codon 30 of *bvgA* to codon 1 of *fhaB*) ligated to another 500bp fragment (corresponding to codons 71-238 of *fhaB*) of RB50 and ligating this ~1kb insert into the MCS of pEG7S.

p∆βhelix$_{fhaB}$ was created by PCR amplifying one 501 bp fragment (corresponding to codons 218-385 of *fhaB*) ligated to another 501 bp fragment (corresponding to codons 1979-2146 of *fhaB*) of RB50 and ligating this ~1kb insert into the MCS of pSS4245.

p∆TPS_{fhaB} was created by PCR amplifying one 460 bp fragment (corresponding to nt within codons 413-567 of *fhaB*) ligated to another 523 bp fragment (corresponding to nt within codon 3 of *fhaB* to codon 30 of *bvgA*, including 426bp of intergenic region) from RB50 and ligating this ~1kb insert into the MCS of pSS4245.

pX9∆P_{fimA} was created by PCR amplifying one 550bp fragment (corresponding to codons 1-183 of *fimA*) ligated to another 500 bp fragment (corresponding to 16 bp 3' to the *fhaB* STOP codon through codon 30 of *bvgA*) of RBX9 and ligating this ~1kb insert into the MCS of pSS4245.

pBam was created by PCR amplifying the *fhaB-bvgAS* intergenic region (corresponding to the 426 bp between each ATG) of RBX9 and ligating this fragment into the MCS of pEG7.

pBamR was created by digesting pBam with BamHI (where BamHI cut sites flanked the insert), ligating, and screening transformed clones for the reverse orientation relative to pBam.

pGFLIP-$P_{bvgA\text{-short}}$ was created by PCR amplifying the *fhaB-bvgAS* intergenic region (426bp) from RB50 and inserting this fragment into the MCS of pGFLIP with the *bvgAS* promoters driving *flp.*

pGFLIP-$P_{bvgA\text{-long}}$ was created by PCR amplifying a 1626 bp fragment including nt corresponding to codon 400 of *fhaB* through the ATG of *bvgA* in RB50 and inserting this fragment into the MCS of pGFLIP with the *bvgAS* promoters driving *flp.*

miniTn7-*flaAgfp* was created by PCR amplifying 500 nt 5' to the *flaA* ATG in RB50 and ligating this fragment into the MCS of pUC18T-miniTn7T-Km. To the resulting plasmid, we ligated sequences encoding promoterless *gfp* from miniTn7T-*kan-gfp* pUC such that the *flaA* promoter drives *gfp*.

pCW103 was created by PCR amplifying sequence encoding an HA-tag flanked by homology region from *batB* including -300 nt from ATG to codon 55 and codons 56-216 and ligating this insert into the MCS of pSS4245.

References

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