Bistable virulence gene expression in *Pseudomonas aeruginosa* requires positive feedback of a LysR-type transcription activator

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Abstract

Bistable gene expression can generate phenotypic diversity in clonal populations of bacteria. Here we uncover a bistable switch in the opportunistic human pathogen *Pseudomonas aeruginosa*. This switch controls the expression of a small subset of genes including *aprA*, which encodes the virulence factor alkaline protease. We present evidence that bistable expression of *bexR* (*b*istable *ex*pression *r*egulator), which encodes a LysR-type transcription activator, mediates this switch. In particular, using DNA microarrays, quantitative RT-PCR analysis, chromatin immunoprecipitation and reporter gene fusions we identify genes directly under the control of BexR and show that these genes are bistably expressed. Furthermore, we present evidence that *bexR* is itself bistably expressed and that its expression is positively autoregulated. Finally, using single-cell analysis of a *GFP* reporter fusion, we present evidence that this positive autoregulation of *bexR* is necessary for bimodal expression of the regulon. Our findings reveal a previously undescribed bistable switch that controls virulence gene expression in *P. aeruginosa*, and suggest that it may be mediated, at least in part, by positive feedback of a LysR-type transcription activator.

BexR controls bistable expression of the PA1202 operon

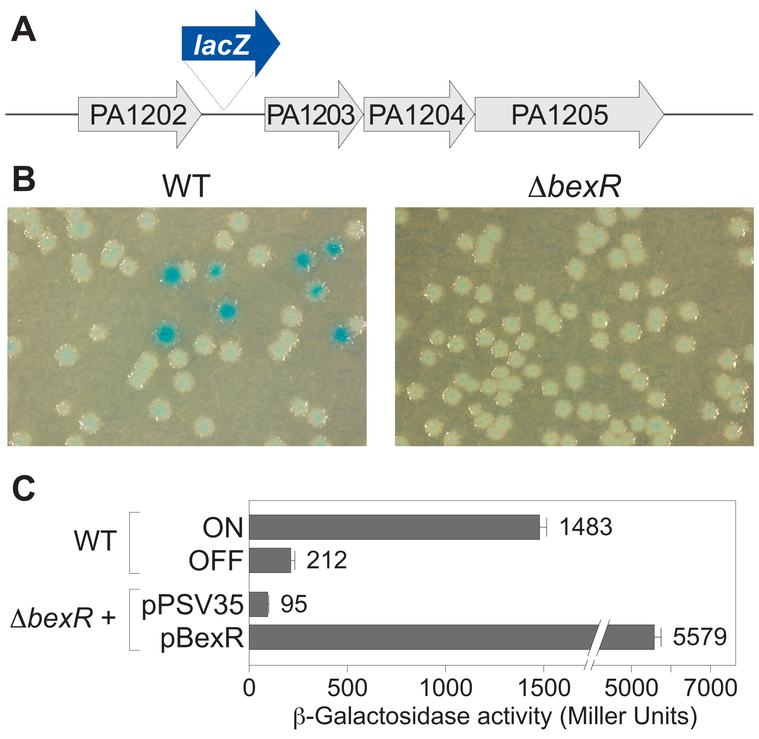

Figure 1. The PA1202 operon exhibits bistable expression in *P. aeruginosa* PAO1 in the presence of *bexR*. **(A)** Schematic of *PA1202 lacZ* reporter strains. **(B)** Phenotypes of wild-type and Δ*bexR PA1202 lacZ* reporter strains when plated on LB agar containing X-Gal. **(C)** Quantification of *PA1202 lacZ* expression in wild-type cells, cells without *bexR* and cells overexpressing *bexR*.

BexR regulates bistability in a clinical *P. aeruginosa* isolate

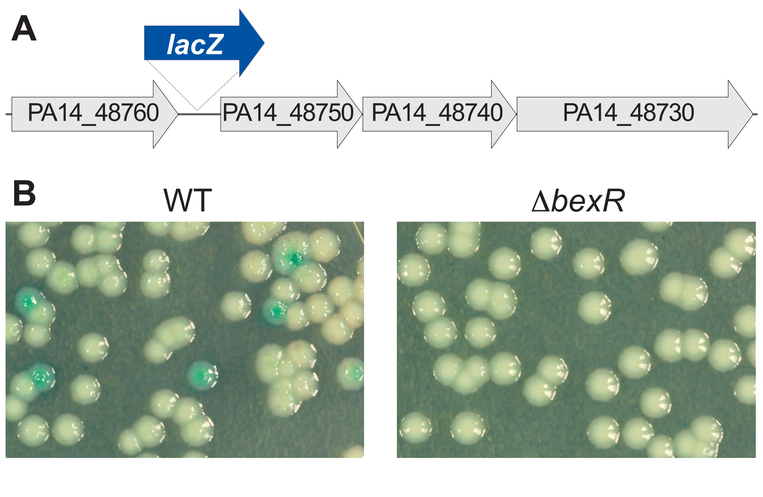

Figure 2. The PA1202-orthologous PA14_48760 operon also exhibits *bexR*-dependent bistability in *P. aeruginosa* PA14. **(A)** Schematic of *PA14_48760 lacZ* reporter strains. **(B)** Phenotypes of wild-type and Δ*bexR PA14_48760 lacZ* reporter strains when plated on M63 agar containing X-Gal. **(C)** Quantification of *PA14_48760 lacZ* expression in wild-type cells and cells without *bexR*.

BexR positively regulates expression of a diverse set of genes, including *aprA*, a known virulence factor

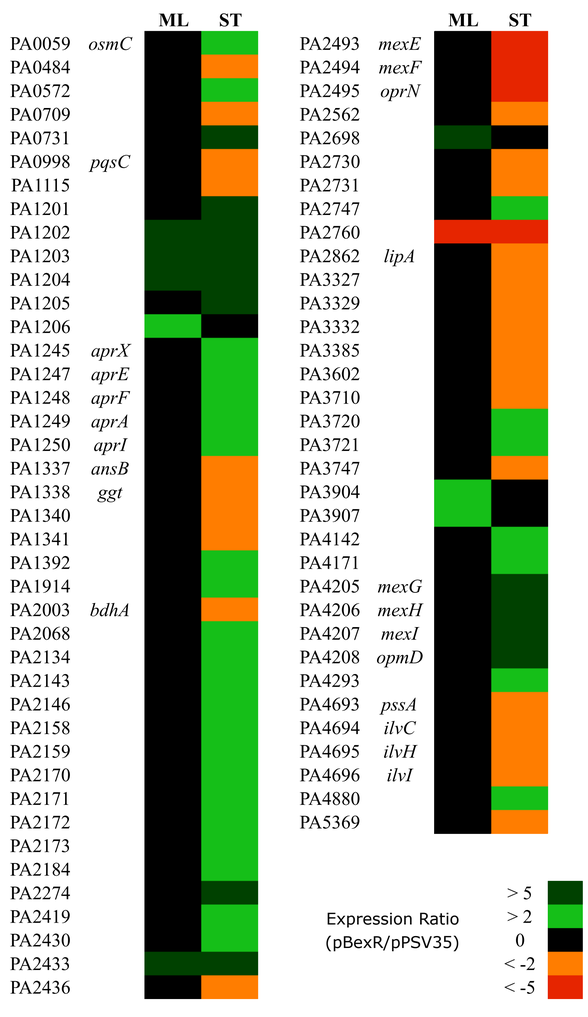

Figure 3. Microarray analysis of the BexR regulon. PAO1 Δ*bexR* was grown to both mid-logarithmic (ML) and stationary (ST) phase with either empty vector or *bexR*-overexpression vector. RNA was isolated and analyzed by microarray.

BexR positively regulates its own transcription and is itself bistably expressed

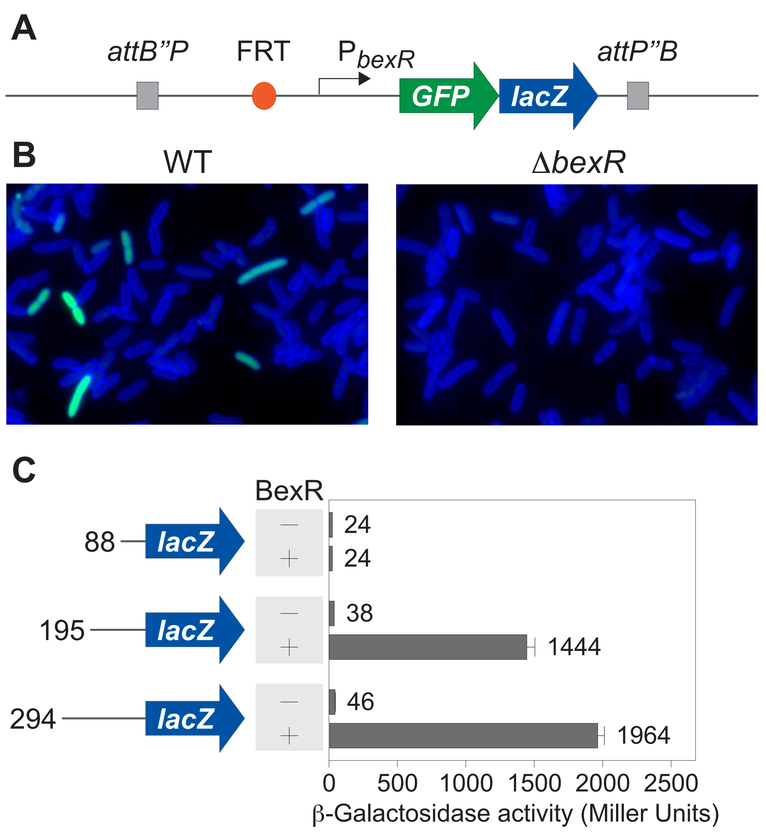

Figure 4. *bexR* is bistably expressed in a *bexR*-dependent manner in PAO1. **(A)** Schematic of reporter construct stably integrated in single copy in P_{bexR}-*GFP-lacZ* and P_{bexR}-*lacZ* reporter strains. **(B)** Micrographs of fluorescent wild-type and Δ*bexR* P_{bexR}-*GFP-lacZ* reporter cells stained with the membrane dye TMA-DPH. **(C)** Quantification of P_{bexR}-*lacZ* expression using varying lengths of *bexR* promoter DNA in cells without *bexR* and cells overexpressing *bexR*.

BexR-regulated transcripts vary between the OFF and ON states

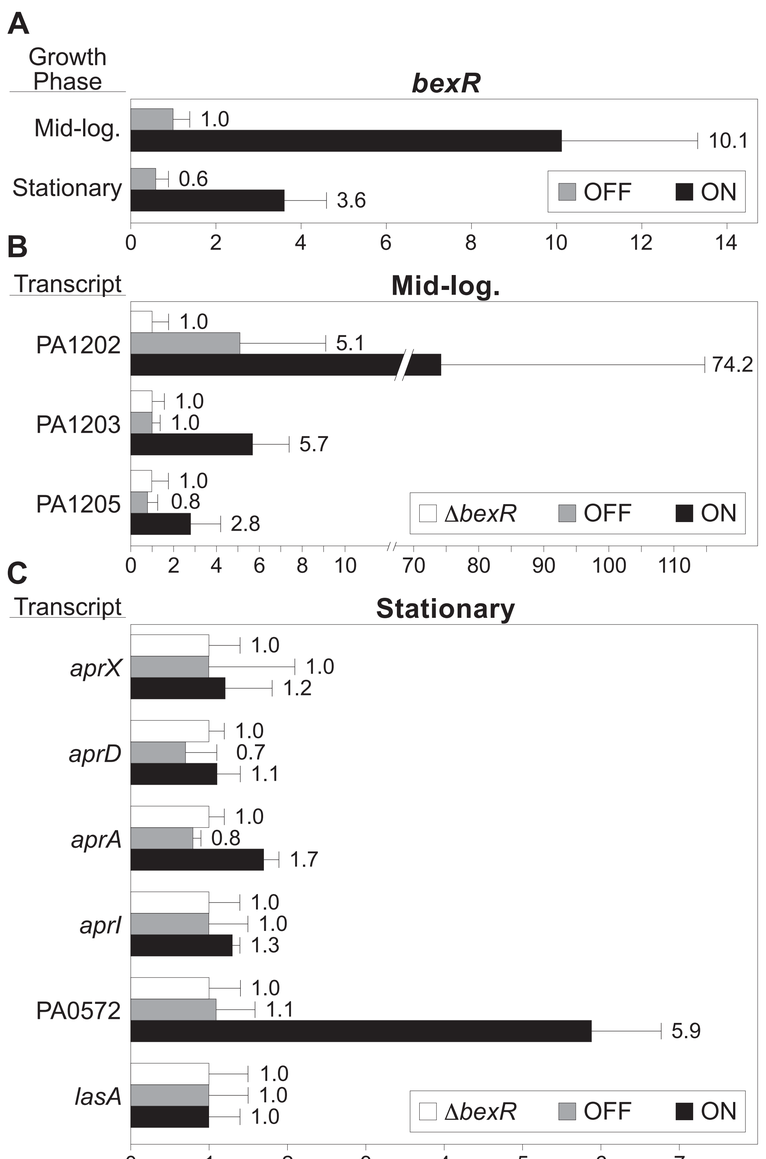

Figure 5. mRNA content of wild-type and Δ*bexR* P_{bexR}-*lacZ* PAO1 reporter strains at both mid-logarithmic and stationary phase. Transcripts were quantified by real-time RT-PCR. **(A)** Relative quantity of *bexR* transcript at mid-logarithmic and stationary phase. **(B)** Relative quantities of PA1202 operon transcripts in mid-logarithmic phase. **(C)** Relative quantities of quorum-regulated transcripts in stationary phase. *lasA* is included as a non *bexR*-regulated control.

BexR acts at the promoters of target genes

Figure 6. BexR co-immunoprecipitates the promoters of target genes. A VSV-G epitope tag was fused to *bexR* at its native locus in PAO1 *PA1202 lacZ*. This epitope tag fusion was shown to retain partial function by β-Galactosidase assay (data not shown). Chromatin immunoprecipitation (ChIP)-enriched DNA was quantitated by real-time PCR as compared to the PA2155 promoter as a non-binding control.

Positive feedback of *bexR* is required for bistability

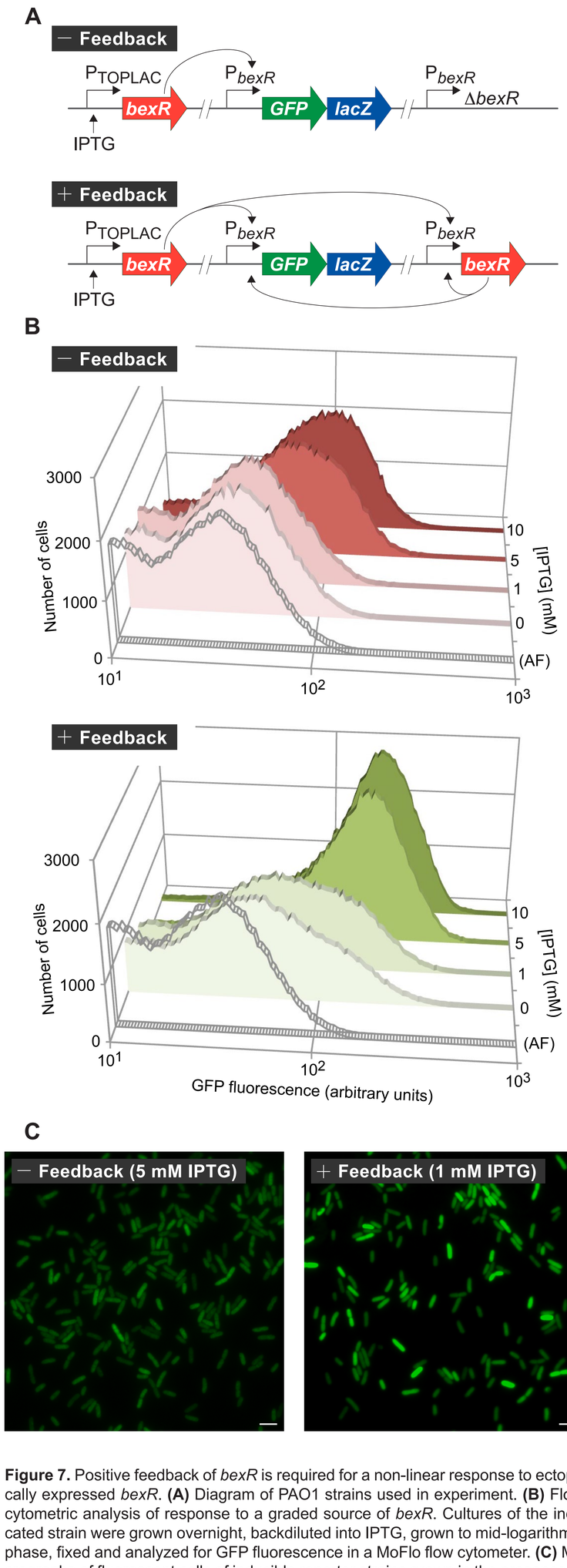

Figure 7. Positive feedback of *bexR* is required for a non-linear response to ectopically expressed *bexR*. **(A)** Diagram of PAO1 strains used in experiment. **(B)** Flow cytometric analysis of response to a graded source of *bexR*. Cultures of the indicated strain were grown overnight, backdiluted into IPTG, grown to mid-logarithmic phase, fixed and analyzed for GFP fluorescence in a MoFlo flow cytometer. **(C)** Micrographs of fluorescent cells of inducible reporter strains grown in the presence of the indicated concentration of IPTG.

Model: Cell-to-cell variability in BexR levels activates a positive feedback loop, resulting in switch to stable ON state in some cells

Figure 8. A model for the switch to the BexR-ON state. Wild-type *P. aeruginosa* is hypersensitized to levels of BexR by virtue of positive feedback at the *bexR* locus. Cell-to-cell variability in basal *bexR* expression results in some cells stochastically reaching a threshold concentration of BexR at which this positive feedback loop is engaged by BexR binding to its own promoter and activating transcription. At this point, the BexR-ON state is maintained by direct positive feedback. Transcription of downstream genes such as *aprA*, PA0572, and those of the PA1202 operon is upregulated in the BexR-ON state by BexR binding at their promoters and directly activating transcription.

Acknowledgements

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