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 (bistable expression regulator), 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 bistable 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

BexR positively regulates its own transcription and is itself bistably expressed

BexR acts at the promoters of target genes

Figure 7. The PA1202 orthologue PA14_48760 operon also exhibits bistable-dependent bistability in P. aeruginosa PA14. (A) Schematic of PA14_48760 lacZ reporter strains. (B) Expression levels of wild-type and JlexR PA14_48760 lacZ-reporter strains when plated on LB agar containing X-Gal. (C) Quantitation of PA14_48760 lacZ expression in wild-type cells and cells without bexR.

Model: Cell-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 hyperactivated at levels of BexR by virtue of positive feedback at the bexR locus. Cell-to-cell variability in basal bexR expression results in some cells 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 the PA1202 operon is upregulated in bistable cells and cells without bexR.

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Figure 6. Positive feedback of bexR is required for a non-linear response to ectopically expressed bexR. (A) Diagram of PA0570 strains used in experiment. (B) Flow cytometric analysis of a reporter plasmid with the lacZ gene fused to bexR. The plasmid is linearized in the bistable ON state by BexR binding to its own promoter and activating transcription.

Figure 5. BexR co-immunoprecipitates with the promoters of target genes. A V5-His epitope tag was fused to bexR at its native locus in PA01. PA01:bexRΔ2. 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.

Figure 4. Relative number of transcripts at the bexR locus. (A) Quantitation of bexR transcripts in wild-type cultures and cultures without bexR. (B) Quantitation of bexR transcripts in wild-type cultures and cultures without bexR. (C) Quantitation of bexR transcripts in wild-type cultures and cultures without bexR.

Figure 3. Schematic of reporter construct stably integrated in single copy in PA01. (A) Schematic of PA01-orthologue reporter strains. (B) Phenotypes of wild-type and bexR-PA01-orthologue lacZ reporter strains when plated on LB agar containing X-Gal. (C) Quantitation of PA01-orthologue lacZ expression in wild-type cells and cells without bexR.

Figure 2. Semi-quantitative analysis of the BexR regulon. PA01::JlexR 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.

Figure 1. The PA1202 operon exhibits bistable expression in P. aeruginosa PA01 in the presence of bexR. (A) Schematic of PA1202 lacZ reporter strains. (B) Phenotypes of wild-type and JlexR PA1202 lacZ reporter strains when plated on LB agar containing X-Gal. (C) Quantitation of PA1202 lacZ expression in wild-type cells, cells without bexR and cells overexpressing bexR.

Cellulo-cell variability in basal bexR levels activates a positive feedback loop, resulting in switch to stable ON state in some cells with higher bexR expression.