H-NS-like Proteins in *Pseudomonas aeruginosa* Coordinately Silence Intragenic and Antisense Transcription

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Abstract

Background: The H-NS-like proteins MvaT and MvaU act coordinately as global repressors in *Pseudomonas aeruginosa* by binding to AT-rich regions of the chromosome, which include horizontally acquired genes and numerous virulence factors. Although cells can tolerate the loss of either protein, identifying their combined regulatory effects has been challenging because the loss of both proteins is lethal due to induction of the prophage Pf4 and subsequent superinfection of the cell. Although in other bacteria, H-NS promotes cellular fitness by inhibiting intragenic transcription from AT-rich target regions, preventing them from sequestering RNA polymerase, a role for MvaT and MvaU in repressing transcription from intragenic promoters has not been demonstrated.

Methods: Here we utilise a parental strain that cannot be infected by Pf4 phage to identify the combined MvaT and MvaU regulon. RNA-seq was utilised to identify genes differentially expressed in cells lacking MvaU or both MvaU and MvaT. ChIP-seq was utilised to identify genes directly regulated by MvaT and MvaU in wild-type cells. Further, ChIP-seq was performed in cells of the parental strain and cells lacking both MvaT and MvaU to map genome-wide σ^{70}-dependent promoters that were active in the presence or absence of both H-NS-like proteins.

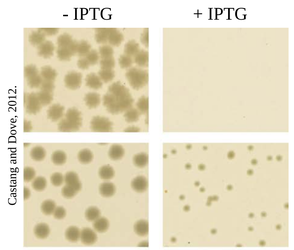
Results: We demonstrate that the loss of both MvaT and MvaU, but not MvaU alone, leads to increased sense, antisense, and intragenic transcription from loci directly controlled by these proteins. We further show that the loss of MvaT and MvaU leads to a striking redistribution of RNA polymerase containing σ^{70} to those genomic regions vacated by these proteins.

Conclusion: Our findings suggest that the ability of H-NS-like proteins to repress intragenic transcription is a universal function of these proteins and describe a second mechanism by which MvaT and MvaU may contribute to the growth of *P. aeruginosa*.

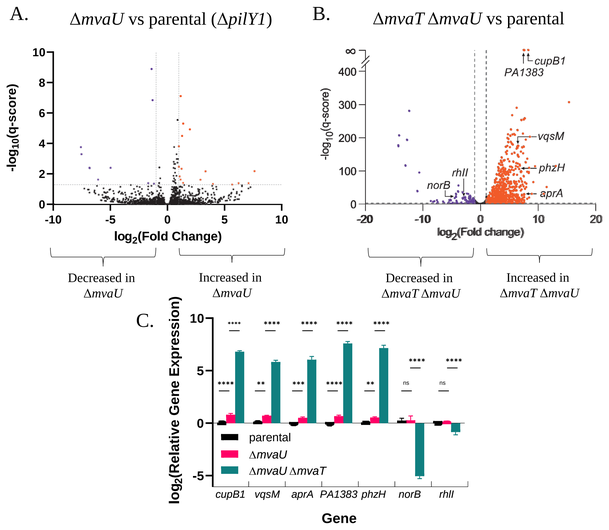
Background

- The DNA-binding global repressor proteins MvaT and MvaU nucleate on DNA, oligomerise along large stretches of DNA, and form DNA-bridges
- Cells cannot tolerate loss of both MvaT and MvaU because they repress expression of endogenous prophage Pf4 genes
- Deletion of the receptor for phage Pf4 (*pilY1*) allows cells to survive loss of and MvaT and MvaU by preventing superinfection by the phage
- Prior studies attempted to identify the regulons of MvaT and MvaU, but deletion of either *mvaT* or *mvaU* lead to compensatory changes in protein levels of the other, confounding data interpretation

Cells tolerate loss of MvaT and MvaU in the absence of *pilY1*



Loss of both MvaT and MvaU results in marked changes in transcription

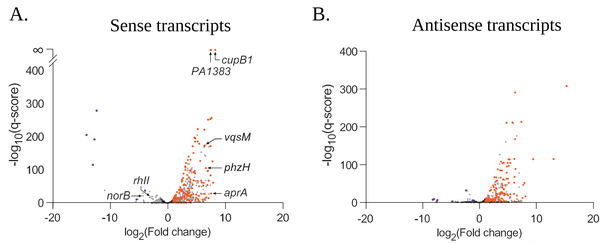


A. Volcano plot of RNA-seq data shows that only 15 genes are differentially expressed in Δ*mvaU* compared to the parental (Δ*pilY1*) with statistical significance (q>0.05, |fold change| >2).

B. Loss of both MvaT and MvaU leads to differential expression of 1407 transcripts, including 535 genes, representing 10% of all annotated genes.

C. RNA-seq results are validated independently by qRT-PCR for the genes indicated with an arrow (B). *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001

Loss of MvaT and MvaU results in global changes in sense and antisense transcription

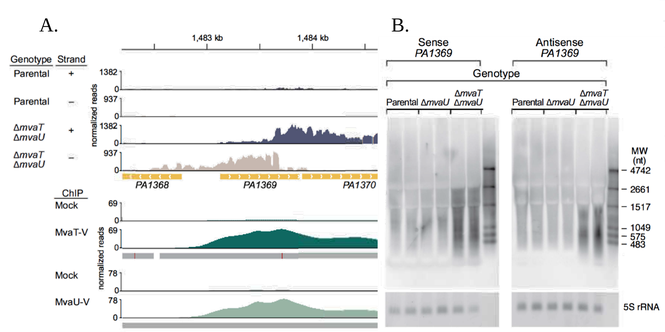


RNA-seq in Δ*mvaT* Δ*mvaU* cells compared to the parental strain coupled with ChIP-seq with MvaT and MvaU in otherwise wild-type cells, which identified loci associated with each protein, shows:

A. 1/5 of differentially expressed sense transcripts in Δ*mvaT* Δ*mvaU* cells are direct targets of MvaT and MvaU (orange and blue); indirect targets are shown in grey.

B. 408 antisense transcripts are expressed in Δ*mvaT* Δ*mvaU* cells, with 1/5 of the loci encoding these transcripts directly associated with MvaT and MvaU.

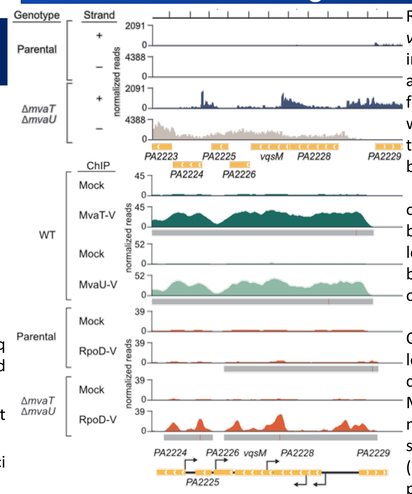
Loss of MvaT and MvaU alters sense and antisense transcription (continued)



A. RNA-seq reads at the *PA1369* locus show increased sense (blue) and antisense (beige) transcripts, which appear to arise from within the gene's coding sequence. ChIP-seq with MvaT and MvaU in otherwise wild-type cells, which identified loci associated with each protein, show that areas with increased transcription in Δ*mvaT* Δ*mvaU* cells compared to the parental strain are direct targets of MvaT and MvaU, respectively.

B. Increased sense and antisense transcription is seen at the PA1369 locus only in the absence of both MvaT and MvaU by Northern Blot. Probes designed to hybridise with sense transcripts arising from *PA1369* DNA (left) and antisense transcripts (right) were used to detect their respective targets in total RNA from the parental strain, cells lacking MvaU, and cells lacking both MvaT and MvaU that are separated by electrophoresis. 5S rRNA staining is shown as a loading control.

Loss of MvaT and MvaU increases intragenic transcription

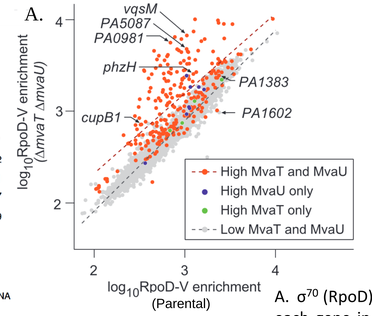


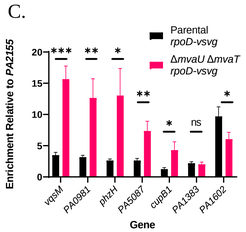
RNA-seq reads at the *PA2228-vqsM-PA2226* operon show increased sense (beige) and antisense (blue) transcripts arising from within the genes. ChIP-seq with MvaT and MvaU shows that the operon is a direct target of both repressors.

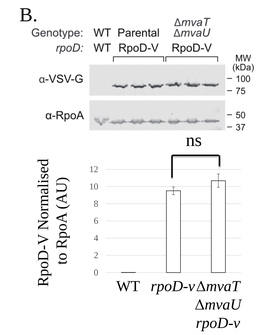
σ subunits of RNA polymerase can be used as a proxy for promoter location or transcription start site because they associate with DNA only during promoter binding.

ChIP-seq with σ^{70} (RpoD) shows localisation within genes only in cells lacking both MvaT and MvaU. Transcription start site mapping using 5'-RACE shows several sense and antisense sites (arrows) that correlate with σ^{70} peaks.

Loss of MvaT and MvaU results in genome-wide redistribution of RNA polymerase





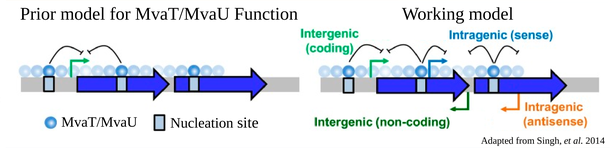


A. σ^{70} (RpoD) ChIP-seq enrichment scores for each gene in the parental and Δ*mvaT* Δ*mvaU* strain are shown as individual data points. Direct targets of both MvaT and MvaU are shown in red while genes that targets for neither MvaT nor MvaU are shown in grey. Genome-wide, there is increased association of the σ^{70} subunit of RNA polymerase with direct targets of MvaT and MvaU, consistent with observed increased sense, antisense, and intragenic transcription,

B. Deletion of *mvaT* and *mvaU* does not alter σ^{70} protein abundance, therefore increased association of σ^{70} with MvaT/U targets may titrate away from non-target genes.

C. Validation of ChIP-seq results by ChIP-qPCR. Indeed, σ^{70} is titrated away from the non-MvaT/U target PA1602 in the Δ*mvaT* Δ*mvaU* strain compared to the parental.

Discussion



Adapted from Singh, *et al.* 2014

Future Directions

H-NS proteins are proposed to repress transcription by a number of mechanisms, including occluding target DNA from RNA polymerase and recruitment of NusG and transcription terminator Rho.

- Evaluate loss of NusG and/or Rho and the effect on repression of spurious intragenic transcription.

Acknowledgments

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