Genes repressed after DNA damage in *Acinetobacter baumannii* are co-regulated by UmuDAb and DdrR

Mollee Flannigan, Xiaohong Li, Eric Rouchka, Deborah Cook, & Janelle Hare, Mentor

Department of Biology and Chemistry, College of Science, Morehead State University
Department of Biochemistry and Molecular Genetics, University of Louisville

ABSTRACT

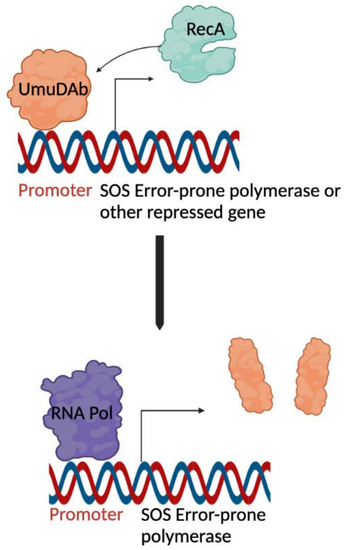
UmuDAb and DdrR coregulate error-prone polymerases in the multi-drug resistant opportunistic pathogen, *Acinetobacter baumannii*, by repressing polymerase expression until after DNA damage. New evidence indicates that these proteins may also regulate other genes that are repressed following DNA damage. We performed an *in silico* analysis of RNA-Seq data from wild-type, *ddrR*, and *umuDAb* mutant strains to examine the expression levels of genes repressed after DNA damage. We used two different algorithms to analyze Cuffnorm- and HTSeq normalized gene counts. This analysis revealed nineteen (CuffDiff) or twenty-nine (DESeq2) genes repressed in wild-type cells that were derepressed after DNA damage in either one or both of the mutant strains. The proteins encoded by these genes include an induced acetoin metabolism operon, a putative YfbU family member (often required for MazF-mediated cell death after DNA damage), RlpA (a septal ring lytic transglycosylase), and a putative cold-shock protein. We carried out RT-qPCR verification of the RNA-Seq data and found that these genes are dysregulated after DNA damage, indicating DdrR and UmuDAb's regulatory functions. Upon completion of RT-qPCR, we will construct strains containing mutations in these genes to test if DdrR and UmuDAb co-regulate these repressed genes. This will aid us in our understanding of how their downregulation may be involved in the pathogen's response to DNA damage-induced stress.

OBJECTIVES

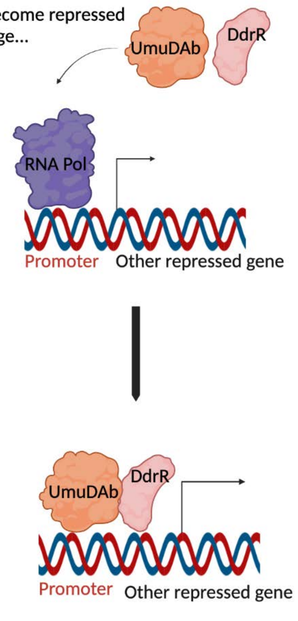
- Use *in silico* transcriptome analysis and RT-qPCR of wild-type (WT), *umuDAb*, and *ddrR* mutants to determine if UmuDAb and/or DdrR are required for the repression of certain genes after DNA damage.
- Explore how UmuDAb and DdrR co-regulate these genes that are no longer repressed in the mutant strains.

SIGNIFICANCE

A. baumannii SOS response system after DNA damage...



Certain genes become repressed after DNA damage...

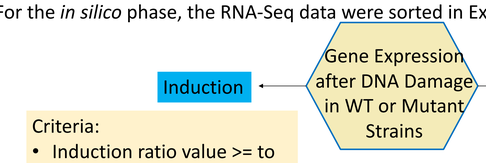


How does DdrR assist UmuDAb in its repression functions? What is the role of the genes that are repressed after DNA damage?

Created in BioRender.com

METHODS

For the *in silico* phase, the RNA-Seq data were sorted in Excel based on specific criteria.[1]:



Criteria:
- Induction ratio value >= to 2.0
- q-value < 0.05
- 152 genes were found to be induced with these criteria[1]

Criteria:
- Induction ratio value <= to 0.5
- q-value < 0.05

RT-qPCR verification experiments were carried out to confirm gene dysregulation.
RT primers were designed for:
- dihydrolipoamide dehydrogenase (*A1S_1702* and *A1S_1703*, denoted 1702-3)
- YfbU family protein (*A1S_3858*)
- RlpA (*A1S_2317*)
- cold shock protein (*A1S_1228*)

RESULTS

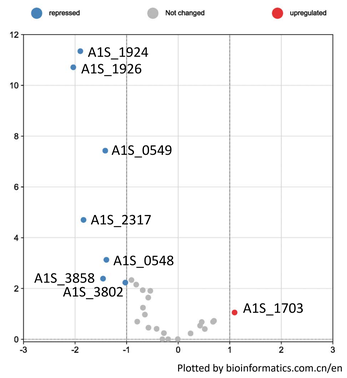
RNA-Seq Analysis Revealed...

Plotted by www.bioinformatics.com.cn/en

- 434 genes were repressed in total[1]
- 19 genes were no longer repressed in both mutant strains, meaning that both UmuDAb and DdrR may be required for their repression.
- 7 genes were no longer repressed only in the *umuDAb* mutant.
- 3 genes were no longer repressed only in the *ddrR* mutant.

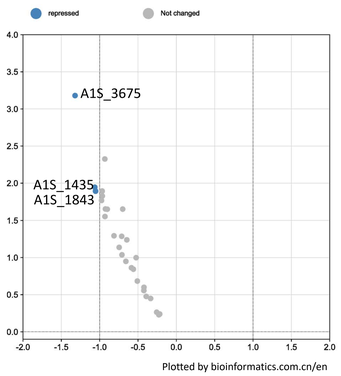
Repressed Genes Dysregulated in the:

***ddrR* mutant**



Plotted by bioinformatics.com.cn/en

***umuDAb* mutant**

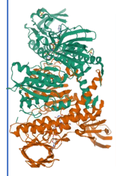


Plotted by bioinformatics.com.cn/en

7 genes that, without DdrR, are repressed (blue dots) under DNA damaging conditions. Conversely, only one genes was upregulated (red dot) under the same conditions.

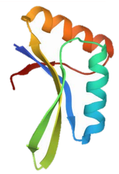
3 genes that, without UmuDAb, are repressed (blue dots) under DNA damaging conditions. Conversely, no genes are upregulated under the same conditions.

RT-qPCR Verification of selected genes revealed...



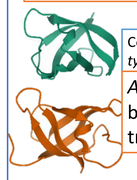
Dihydrolipoamide dehydrogenase, *Escherichia coli*[2] (*A1S_1702, A1S_1703*)

A1S_1702/A1S_1703 were chosen because of their involvement in metabolic activity.



SPOR domain of RlpA, *Pseudomonas aeruginosa*[3] (*A1S_2317*)

A1S_2317 was chosen because of its role in cell division

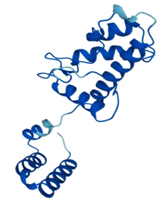


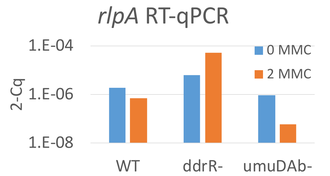
Cold shock protein CspE, *Salmonella typhimurium*[4] (similar to *A1S_1228*)

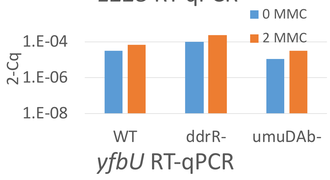
A1S_1228 was chosen because of its putative role in transcription

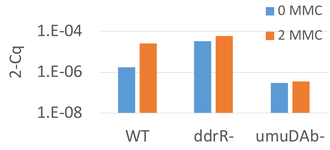
YfbU, *E. coli* (*A1S_3858*)

A1S_3858 was chosen because of its role in the cellular death pathway.









	Transcriptome Data			RT-qPCR Verification		
Gene	**WT**	***ddrR***	***umuDAb***	**WT**	***ddrR***	***umuDAb***
A1S_1702-3	DOWN	UP	UP	DOWN	DOWN	UP
A1S_2317 (RlpA)	DOWN	DOWN	UP	DOWN	UP	UP
A1S_3858 (YfbU)	DOWN	DOWN	UP	UP	UP	UP
A1S_1228	DOWN	UP	DOWN	UP	DOWN	UP

Downregulation (DOWN) vs. upregulation (UP) of genes post-DNA damage in transcriptome and RT-qPCR data. Green indicates verification; red is disagreement

CONCLUSIONS

- RT-qPCR verification partially confirmed the dysregulation of the selected genes in the *umuDAb* and *ddrR* mutant strains.
- There are discrepancies between the RNA-Seq data and the RT-qPCR data, most interestingly that the genes associated with YfbU and the putative cold shock protein are not repressed in the wild-type strain after DNA damage as previously believed. This may be due to the generally low gene expression of these two genes, but until we understand their role in *A. baumannii*'s SOS response the cause of this discrepancy is unclear.
- In the future, further RT-qPCR experimentation will be carried out to investigate other genes that were repressed in wild-type after DNA-damaging treatment but were no longer repressed in the treated mutant strains. Then, mutants will be made of candidate genes to determine what roles they may play in *Acinetobacter baumanni*'s DNA damage-induced stress response.

REFERENCES

1. Hare JM, Ferrell JC, Witkowski TA, Grice AN (2014) Prophage Induction and Differential RecA and UmuDAb Transcriptome Regulation in the DNA Damage Responses of *Acinetobacter baumannii* and *Acinetobacter baylyi*. PLOS ONE 9(4): e93861.
2. Chandrasekhar K, Wang J, Arjunan P, et al. (2013). Insight to the interaction of the dihydrolipoamide acetyltransferase (E2) core with the peripheral components in the Escherichia coli pyruvate dehydrogenase complex via multifaceted structural approaches. J Biol Chem. 288(21):15402-15417.
3. Alcorlo, M., Dik, D.A., De Benedetti, S. *et al.* Structural basis of denuded glycan recognition by SPOR domains in bacterial cell division. *Nat Commun* **10,** 5567 (2019). Morgan HP, Wear MA, McNae I, Gallagher MP, Walkinshaw MD. (2009). Crystallization and X-ray structure of cold-shock protein E from Salmonella typhimurium. Acta Crystallogr Sect F Struct Biol Cryst Commun 65(Pt 12):1240-1245.

ACKNOWLEDGMENTS

This work funded by: NIH grant 2R15GM085722-03A1 and NIH grant 2P20RR016481-09.
Thank you to the MSU Honors Program for continued support of our endeavors.