

64. Uranium exposure elicits a multifaceted stress response in *Caulobacter crescentus*

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Project Goals. The oxidized form of uranium (U(VI)) predominates in oxic environments and poses a major environmental threat due to its high toxicity and mobility.

Microorganisms play an important role in governing U speciation and transport in the environment and represent a promising U bioremediation platform. Although mechanisms of microbial-mediated U(VI) immobilization have been well-studied, information regarding the specific mechanisms involved in U(VI) toxicity remains limited. A major aim of our project is to understand the mechanisms by which the obligate aerobic *Caulobacter crescentus* mitigates U toxicity in the environment. To this end, we have applied a functional genomics approach to identify genomic elements critical for uranium tolerance.

To gain insight into the mechanism of U(VI) toxicity, we characterized the stress response pathways critical for uranium tolerance in the obligate aerobic *Caulobacter crescentus* that is of interest for bioremediation because of its ability to mineralize U(VI) under aerobic conditions. RNA-seq and proteomics were used to identify upregulated stress response pathways during exposure to toxic concentrations of U, with the contribution of such pathways to U tolerance assessed by gene deletions. Among the differentially expressed genes include several global stress regulators and regulon members, including those responding to ROS, DNA damage, heat shock and extracytoplasmic stresses. In particular we found that a *recA* deletion mutant was hypersensitive to uranium exposure. Intriguingly this phenotype was independent of SOS induction since a *recA* mutant lacking the ability to induce the SOS response did not exhibit increased U sensitivity, suggesting that the homologous recombination function of RecA is critical for U tolerance. Additionally, the loss of ClpAP and DegP protease function also significantly reduced growth rate during U exposure, as did a σ^{32} partial loss of function allele. Consistent with an oxidative stress response, U shifted the GSSG/GSH redox couple towards the oxidized state in a concentration dependent manner and the loss of key antioxidant enzymes, including superoxide dismutase (*sodB*), catalase/peroxidase (*katG ahpF* double mutant) and glutathione reductase (*CCNA_02387*) reduced U tolerance. Finally, our data revealed a link between uranium toxicity and cell cycle progression, since nearly the entirety of the *Caulobacter* cell cycle machinery was downregulated and protein levels of the two master cell cycle regulators, CtrA and DnaA, were depleted upon exposure to U. Together, our data suggest that uranium exerts a multifaceted toxicity by damaging DNA, disrupting cytoplasmic and extracytoplasmic protein homeostasis and by causing oxidative stress.

References

1. Hu P, Brodie EL, Suzuki Y, McAdams HH, Andersen GL. 2005. Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. *Journal of bacteriology* 187:8437-8449.