

## **Characterization of a Metal-induced Sensory Transduction System in *Caulobacter crescentus***

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**Project Goals: Depleted uranium (U) is a widespread environmental contaminant that poses a major threat to human health. In contrast to humans and animals where trace amount of U can cause damage to kidneys, liver and heart, it is well known that some bacteria can tolerate high levels of U and influence its mobility and bioavailability in the environment. As a non-pathogenic bacterium, *Caulobacter crescentus* is an attractive bioremediation candidate due to its high tolerance to heavy metals, and its ability to mineralize U by facilitating uranium phosphate precipitation. Our goal is to decipher the physiological basis for U tolerance in *C. crescentus*, and provide insight into the effect of aerobic bacteria on U biogeochemistry and assess the utility of them in biomineralization applications.**

Maintaining homeostasis for biologically required metal ions and detoxifying the incursion of toxic metal ions require extensive regulatory and response machineries. These processes are essential for cell survival especially under conditions of metal deprivation or overload. Earlier work from Dr. L. Shapiro group at Stanford U. identified a small periplasmic protein UrcA in *Caulobacter crescentus* whose expression level increases in responding to the presence of U. To further understand the specificity of this U response, we examined the change in expression of *urcA-lacZ* in responding to several metals including Ag, Cu, Ni, Cd, Co, Cr, and Zn, along with several other stress conditions. We observed that besides U, *urcA* expression was also induced sharply by Zn. We do not yet fully understand why uranium and Zn induce *urcA* expression; Through RNA-seq and proteomic analyses, we found both U and Zn cause cell envelope stress in *Caulobacter*.

To identify regulators involved in controlling the expression of *urcA*, we performed a genetic screen to examine disruption of which gene affects *urcA-lacZ* expression. A total of 60,000 transposon mutants were screened and we identified a previously uncharacterized two-component system, named *urcS/urcR*, that serves as a direct activator of *urcA*. Inactivation of either *urcS* or *urcR* completely abolished the *urcA-lacZ* expression in the presence of Zn. Furthermore, direct binding of cytoplasmic response regulator UrcR with DNA upstream of *urcA* was confirmed by an electrophoretic mobility shift (EMSA) assay.

Besides the *urcS/R* two component system that is a direct activator of *urcA*, we found three other proteins that are indirect repressors of *urcA*, all of which act through *urcS/R*. These include an ABC transporter with a peptidase domain (*pepN*), a conserved membrane protein and two general stress regulators belong to the MarR family. Genetic disruption of any of these proteins caused constitutive expression of *urcA*, independent of Zn or U. Zn addition further increased the *urcA* expression in these mutant backgrounds however, suggesting an alternative mechanism for Zn-induced *urcA* expression

independent of these repressors. In particular, disruption of key amino acids of the peptidase domain of the ABC transporter-pepN protein or the ATP-binding domain of its neighboring ATPase caused upregulation of *urcA*, suggesting a fully functional protein is required to keep *urcA* at the off state in the absence of the environmental signal (U or Zn).

To identify others genes belong to the UrcR regulon other than *urcA*, we performed RNA-seq analysis comparing genomic-wide mRNA expression profile between wild type and the *urcR* null mutant with or without Zn. Our results showed that UrcR is a global regulator of 165 ORFs, the majority of which encode non-cytoplasmic proteins and contain the previously predicted m\_5 motif (metal specific genes). Besides proteins of unknown functions, UrcR also regulate 9 putative non-cytoplasmic peptidases and a few multi-drug efflux pumps. Current work focuses on deciphering the nature of the signal(s) generated by metals (Zn or U) that result in *urcA* induction. The signaling pathway identified will help aid the sensing and stress response studies in *Caulobacter* and has important applications for environmental metal detection and remediation.

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