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Global Gene Regulation in *Desulfovibrio vulgaris* Hildenborough

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Desulfovibrio vulgaris Hildenborough (DvH) is an obligate anaerobe and has been used as a model organism for studying the energy metabolism of sulfate-reducing bacterium (SRB). However, experimental data about the transcriptional regulatory networks which are essential for understanding the cellular processes are very limited. In this study, several predicted global regulators are investigated via mutant characterization, transcriptomic assay and their *in vivo* gene regulations using ChIP-chip assay.

CRP/FNR. CRP/FNR regulators are DNA binding proteins function as positive transcription factors. There are four CRP/FNR homologues in the DvH genome (DVU2547, DVU0379, DVU3111, DVU2097). Evidence from other bacteria demonstrated that CRP/FNR regulators function in response to a broad spectrum of intracellular and exogenous signals such as oxidative and nitrosative stress, nitric oxide, carbon monoxide or temperature. Microarray data from DvH shows that their transcript levels are altered in response to nitrate, nitrite, heat shock, and oxygen stresses. To determine the function to the DvH CRP/FNR, knockout mutants for all four CRP/FNR proteins were generated. The mutants will be characterized using various electron donors and acceptors, different stressors, and transcriptomic analysis. To study the global gene regulation by CRP/FNR, recombinant proteins for all four CRP/FNR were obtained and polyclonal antibodies were generated. Immunoprecipitated DNA-protein complexes with specific CRP/FNR polyclonal antibodies will be hybridized to the DvH PCR-amplicon promoter array. And the CRP/FNR binding motif can be identified by computational and experimental approaches.

H_2O_2 stress response. Oxidative stress is one of the most common environmental stressors. Evidences show that DvH cells are aero-tolerant although they are strict anaerobe. But little is known about molecular mechanisms of oxidative stress responses. DvH is one of the few microorganisms that contain both defense systems which are typical for the aerobic (Sod and Kat) and the anaerobic (Rub, Rbr, Rbo etc.) microbes, but their roles remain elusive. In this study, DvH cells were stressed with two different concentrations of H_2O_2 (1 mM, 4 mM) and 5 time-points (30, 60, 120, 240 and 480 min) were used for the transcriptomic analysis. Microarray data demonstrated that higher concentration of H_2O_2 had broader effect on gene expression. The time-points with the greatest gene expression changes are 120 min (485 up and 527 down) and 240 min (750 up and 753 down) for 1 mM and 4 mM of H_2O_2 respectively. Rdl, Rbr2 were up-regulated, which suggest that these two proteins, rather than Rub-Rbo & Rbr suggested by Coulter's *in vitro* experiment data, may play major roles in H_2O_2 stress. Genes in the predicted PerR and FUR regulon were also up-regulated. Some interesting candidates such as DVU3269 (a

hybrid histidine kinase (HK)), DVU3136 (a nitroreductase family protein) etc. were significantly up-regulated., The function of the putative candidates in H_2O_2 stress response are going to be confirmed by other approaches such as knockout mutant analysis.

FUR, PerR and ZUR. FUR, PerR and ZUR are Fur Paralogs in the DvH genome. Microarray data show that they are involved in iron acquisition, acid shock response and oxidative stress etc. The functional analysis of these three global transcription regulators is in progress.