EBS/CMOP Undergraduate Intern Mentoring Opportunity

Project Title: NsrR transcriptional regulation

Context for Project: NsrR is a Fe-S protein that controls transcription in response to nitric oxide (NO). We have previously shown how NO inactivates NsrR repressor activity (class I regulation). We recently found that NsrR exhibits a different mode of transcription regulation (class II regulation) in *Bacillus subtilis*. The project will investigate the mechanism of newly discovered transcription control by NsrR.

Brief Description: Unlike class I NsrR regulation, class II regulation involves NsrR interaction with A+T-rich target DNA with relaxed sequence-specificity, suggesting the importance of DNA topology. Because of this unique characteristic, it is difficult to determine a detailed mechanism of NsrR/DNA interaction in vitro. Therefore, we will carry out the ChAP (chromatin affinity precipitation)-PCR approach to determine NsrR/DNA interaction in vivo. Furthermore, NsrR co-regulates transcription with other transcription factors that also bind A+T-rich DNA. Therefore, we will investigate how binding of one transcription factor affects binding of another transcription factor using strains lacking each transcription factor.

Proposed Outcomes/Broader Impact: Recent studies have revealed that transcription control of a given gene is often mediated through many activators/repressors. The strategy we plan to take would likely uncover how such cooperative regulation by multiple factors determine cell's fate by fine tuning gene expression. The NSF grant supported this project will end on July 31. My PhD student Sushma Kommineni who has worked on this project will graduate this summer. I submitted a Partners-in-Science proposal to M. J. Murdock Charitable Trust to support a high school teacher for two coming summers. I hope that he and the summer intern will conduct the project when Sushma is focusing on her dissertation so that we will be able to gather more data for an NSF proposal in order to continue the research in the next three years.

Proposed timeline (within a 10 week span):

Week 1-2. *B. subtilis* strain construction. We have already constructed strains used for ChAP-PCR. S/he will introduce necessary mutations in these strains. During this period, s/he will learn basic techniques of microbiology and molecular biology, which include sterilization technique, *Escherichia coli/B. subtilis* transformation, transduction, cloning, plasmid/chromosomal DNA preparation, agarose gel electrophoresis.

Week 3-6. S/he will carry out ChAP-PCR experiments using the wild-type and mutant strains. Association of NsrR with several candidate genes will be determined. S/he will learn SDS-polyacrylamide gel electrophoresis, western analysis, PCR, and affinity precipitation techniques.

Week 7. S/he will analyze the data to identify consensus NsrR-target sequence using the MEME software program (a motif-based sequence analysis tool).

Week 8-10. Generate mutations in the identified motif to determine the effect of mutations on NsrR binding and regulation in vivo. S/he will write a report and give presentation both in EBS/CMOP meeting and Zuber/Nakano weekly lab meeting.