OVERVIEW

Bacterial biofilms are a public health threat because they cause chronic and hospital-acquired infections but are resistant to antibiotics. *Failure to characterize the biochemical machinery that drives biofilm dispersal risks missing key targets for treatment of infectious disease*. Although nitric oxide (NO)-triggered biofilm dispersal in *Pseudomonas aeruginosa*, a principal pathogen in cystic fibrosis and hospital-acquired infections, is well documented, *the underlying biochemical processes responsible are not understood*. To *bridge this knowledge gap*, our *long-term goal* is to determine NO signaling mechanisms in bacteria and use them as a basis for developing therapeutic strategies to treat biofilms.

We have previously established NosP (NO sensing protein) hemoproteins, *discovered in our laboratory*, as NO sensors regulating biofilm in *P. aeruginosa* and other bacteria. NosP (PA1975) binds NO with picomolar affinity using a histidine-ligated protoporphyrin IX. NO/NosP regulates the kinase and phosphotransfer activities of NahK (NosP-associated histidine kinase; PA1976) within the GacS/Rsm multikinase network (MKN). The GacS/Rsm MKN integrates signals from many sensor kinases to control RsmA, the master regulator of motility/acute infection v. biofilm/chronic infection in *P. aeruginosa*.

Recently, we have obtained preliminary data consistent with an additional role for NO/NosP/NahK as an "early warning system" for accumulated intracellular NO. Although at low concentrations, NO signaling regulates processes such as quorum sensing and biofilm dispersal, it is cytotoxic at high concentrations due to nitrosylation of DNA and proteins. To combat NO toxicity, *P. aeruginosa* express two NO detoxification pathways, the NO (Nor) pathway and the flavohemoglobin nitric oxide dioxygenase (Fhp) pathway. Expression of Fhp is modulated through its cognate regulator FhpR, as well as two accessory proteins AsrA and PA14_54210. We have preliminary data that support a hypothesis that the Fhp/FhpR NO detoxification pathway is under the control of the NosP/NahK two-component signaling system.

To test our hypothesis, we have designed two specific aims. In Aim 1, we will determine the transcription level of *fhp*, *fhpR*, *asrA* and *PA14_54210* in wild-type, *ΔnosP*, and *ΔnosPΔnahK* strains of *P. aeruginosa* with and without NO. Deletion strains of *fhp* and *fhpR* will also be created to investigate whether the NO-mediated biofilm dispersal phenotype is affected by the loss of this detoxification network. In Aim 2, we will generate *Δfhp* and *ΔfhpΔnosP* mutant strains and determine the NO-mediated biofilm dispersal phenotype to confirm that Fhp/FhpR activity is downstream of NosP/NahK signaling.

Upon completion of these aims, we *expect* to determine the role of NO/NosP/NahK in controlling the Fhp NO detoxification system. The proposal is *innovative* because it challenges the established anaerobic respiration, NO, biofilm, and detoxification. This proposal is *significant* because elucidation of the molecular basis for NO signaling and toxicity in *P. aeruginosa* will define novel antibiotic targets, for which there is a pressing need, especially considering the antibiotic resistance associated with biofilm infections. NO-triggered biofilm dispersal has been widely observed in bacteria, so clinical interventions based on NO signaling have the potential for widespread application, furthering the *significance* of this project.