Specific Aims

The pathogenesis or virulence of Pseudomonas aeruginosa is regulated by a multitude of signaling pathways and environmental factors. In this sea of overlapping networks, molecules and regulatory proteins, our research has focused on transcription factors belonging to the enhancer-binding protein (EBP) family. EBPs are unique among transcription factors, because they interact exclusively with the sigma factor RpoN to activate transcription of target genes. In P. aeruginosa, RpoN regulates one of the largest bacterial gene networks, encompassing more than 600 genes. Functions such as antibiotic resistance, biofilm formation, motility, utilization of host-derived nutrients, and quorum sensing are all governed by EBP-RpoN regulation.

EBPs are the essential machinery of RpoN-mediated transcription. They provide spatial and temporal specificity to this process. Importantly, signals leading to RpoN-mediated transcription are parlayed through EBPs. The signals themselves might be sensed by the EBPs directly or by histidine kinases (HK), which upon receiving the signal, phosphorylate their cognate or partner EBPs. The ‘active’ EBPs interact with RpoN to initiate transcription from target genes. In the case of P. aeruginosa, there are twenty-two EBPs that can interact with RpoN to regulate gene transcription.

Our long-term goal is to develop a strategy to combat P. aeruginosa pathogenesis by specifically antagonizing EBP-RpoN regulation. The overall objective of this proposal is to complete the characterization of EBPs and their partner HKs in P. aeruginosa PAO1. Knowledge of this regulation, especially the signals and target genes, is essential for achieving this long-term goal. As discussed in the Progress Report, we have successfully identified the biological functions and regulation for the majority of EBPs in P. aeruginosa PAO1. Only a few EBPs remain to be characterized. The central hypothesis of this proposal is that EBPs and their partner HKs regulate diverse functions in P. aeruginosa, including nutrient utilization, protein secretion, virulence-factor production and alginate biosynthesis. The rationale for the proposed project is that knowledge of both the biochemistry and overall biology of the EBPs and their partner HKs in P. aeruginosa will provide new inlets and strategies to address the virulence of this human pathogen.

Significance of EBP regulation in P. aeruginosa biology and virulence will be addressed by two specific aims:

Aim 1. Complete the Characterization of EBPs in P. aeruginosa PAO1. The working hypothesis is that each EBP binds to a specific DNA sequence located upstream of its target genes, thereby leading to transcriptional activation, or in some instances, repression of target genes. The approach will be to purify each EBP and measure its DNA-binding specificity via electrophoretic-mobility shift assays (EMSAs). DNA-binding specificities are not known for the EBPs AauR, MifR, DdaR, EatR, PA1663 and PA1945 (the focus of this aim). Furthermore, the biological functions for the EBPs PA1663 and PA1945 are not understood. To shed light into potential target genes and functions, RNA-seq will be performed on △PA1163 and △PA1945 mutants. Follow-up EMSA assays and genetic/biochemical tests will be conducted to confirm the target genes and biological functions of PA1663 and PA1945. The results generated from these experiments will define the last few unknowns regarding EBP functions in P. aeruginosa, and therefore, solidify a fundamental understanding for EBP regulation in this important human pathogen.

Aim 2. Characterization of the HKs AauS, CbrA, DctB, KinB and MifS of P. aeruginosa PAO1. The working hypothesis is that each HK binds to a specific signal, leading to its autophosphorylation and the subsequent phosphotransfer onto its cognate EBP. The approach will be to measure the substrate specificity and kinetics of each HK in vitro. The biochemistry is not known or poorly defined for five, EBP-related HKs: AauS, CbrA, DctB, MifS and KinB (the focus of this aim). To strengthen the quality and reproducibility of these assays, HKs will be assembled into nanodiscs, which offer several advantages over traditional approaches to membrane-protein characterization. Rates for autophosphorylation, ATP hydrolysis and phosphotransfer will be measured for each HK-nanodisc assembly. The results generated from these experiments will definitively demonstrate the substrate specificities of these HKs, and importantly, define the signals regulating these HKs and EBPs in P. aeruginosa.

The expected outcomes of the proposed project will be a complete understanding of EBP regulation in P. aeruginosa. The results generated by the proposed project will have an important, universal, positive impact, because we will finally know how EBPs contribute to gene regulation and the virulence of this pathogen. Knowledge of these regulatory mechanisms will put us in a strategic position to contest P. aeruginosa pathogenesis by attacking EBP-RpoN regulation.