Abstract

Quorum sensing in bacteria has been extensively examined over the past two decades. These studies suggest that the molecular mechanism that governs quorum sensing incorporates niche-specific, environmentally sensitive information in addition to chemosensory information embedded in the signaling molecules- autoinducing peptides (AIPs) or homoserine lactones. One aspect of the quorum sensing mechanism that was examined in the course of this study was the sensitivity and fidelity of the intracellular signal transduction cascade that correlates extracellular information with a cellular response. The Agr quorum-sensing system in *Staphylococcus aureus* provided a good model system to examine this aspect of the mechanism. As individual components of this mechanism had been characterized by other groups earlier, the tools to probe the intracellular cascade existed at the start of this project. This thesis describes the progress made in the course of studies to examine the role of two important intracellular components- AgrC and AgrA. These proteins were examined using a variety of biophysical, biochemical and structural biology techniques.

This thesis is organized as follows:

Chapter1 provides a broad introduction to quorum sensing in bacteria. The molecular mechanism that governs quorum sensing is described in the context of the clinically relevant bacterial pathogen *Staphylococcus aureus* and other pathogenic and non-pathogenic Staphylococci. Previous work in this area has been summarized to provide a background for this study. The later part of this chapter describes the mechanistic features of the intracellular signal transduction cascade triggered by Agr quorum sensing in the context of the biology of this pathogen.

The role of the intracellular AgrC-AgrA complex in the spontaneity of quorum sensing mechanism is described in chapter 2 of this thesis. The crystal structure of the ATP binding domain of AgrC formed a basis for a mechanistic model of the AgrC-AgrA complex. This model provided an insight into the transient interactions of AgrC with AgrA (cognate histidine kinase and response regulator). This mechanistic model was subsequently validated using spectroscopy experiments. These experiments provided an estimate of the binding affinity, specificity and kinetics of the phosphotransfer reaction. Monitoring the downstream effect of this signal transduction from AgrC to AgrA led us to conclude that the

response time for transcriptional re-engineering triggered by the recognition of autoinducing peptides by AgrC is similar in all four *Staphylococcus aureus* Agr types.

The differences between AgrA interactions with different promoters have been suggested to be the primary reason for the divergent responses of Staphylococci to autoinducing peptides. The crystal structures of several AgrA LytTR domain-DNA complexes were determined to examine this aspect in more detail. AgrA-promoter interactions are described in chapter 3 of this thesis with a view to understand the structural basis for promoter selectivity. A comparison between the structures of different AgrA-promoter complexes revealed features that could rationalize the role of this protein as a transcriptional activator.

The role of AgrA phosphorylation in promoter specificity and promoter strength is described in chapter 4. Surface Plasmon Resonance experiments revealed differences between the interactions of phosphorylated and non-phosphorylated AgrA with the P1, P2 and P3 promoter elements. The strength of these promoters was evaluated using green fluorescent protein based reporter assays. These assays, performed in *Staphylococcus epidermidis*, revealed that phosphorylation selectively activates expression from the P3 promoter. This finding is relevant as the expression of virulent genes and toxins is under the control of the P3 promoter.

Chapter 5 summarizes the results described in this thesis. One conclusion was that the response time for quorum sensing did not differ between the different clinical strains of *S. aureus* that were examined in this study. The second finding was that the fidelity of the quorum sensing response was maintained at the level of AgrC-AgrA interactions. In particular we could note that the histidine kinase AgrC failed to activate non-cognate response regulators. Studies on AgrA-promoter interactions, on the other hand, revealed that AgrA acts as an activator by selectively enhancing the occupancy of the RNA polymerase enzyme at sub-optimal promoters. Put together, these observations suggest a role for the quorum sensing mechanism as a switch between the persistent and the virulent phenotypes. One aspect that remains unexplored is the mechanism that assimilates diverse environmental information, such as metabolite concentrations or redox stimuli, to achieve a nuanced response to a quorum stimulus. Another observation that needs to be examined further is the role of mRNA stability in quorum sensing. The suggestion that the RNA helicase CshA selectively regulates intracellular levels of AgrA mRNA is particularly relevant in this

context. The studies reported in this thesis could thus form the basis for directed experiments to explore these aspects of quorum sensing in *Staphylococcus aureus*.