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Dear Members of the Faculty Search Committee,

I would be pleased to be considered for a tenure-track appointment in response to the Aug. 26th announcement in *Nature* for a position in Systems Biology/Microbiology in the Department of Biology and the Biocomplexity Institute at Indiana University. Currently, I am a postdoctoral fellow in the laboratory of Richard Novick at the New York University School of Medicine.

My research interests center around the social lives of bacteria, with particular emphasis on the fascinating social exchanges that occur in the staphylococci. I have recently discovered that cell-cell signaling controls the development of staphylococcal social motility—a cooperative behavior which may be involved in colonization and virulence. As staphylococci are typically thought to be non-motile, the description of social migration is a significant finding. It also forms the foundation for a unique experimental system to study the effects of cooperative and antagonistic cell-cell signaling on staphylococcal behaviors. These behaviors may be undermined by signaling cheaters and defectors, and my research plan attempts to address the biochemical nature and evolutionary significance of these microbial encounters. My goals are to elucidate the molecular mechanisms which control social behaviors, investigate the effects of cheaters and defectors on social cooperation and species diversity, and determine the implications of these social exchanges on pathogenesis and transmission. A feature of my program which may be of particular interest to your department would be the application of genomic-scale comparative analysis to understand how cell-cell signaling influences species diversity. Our understanding of staphylococcal societies may reveal important aspects of multicellular development and organization that could be exploited to prevent bacterial infections.

Prior to my postdoctoral studies, I received my Ph.D. working with the late Robert J. Kadner at the University of Virginia on bacterial two-component signaling. There, I developed my skills in bacterial genetics and biochemistry and applied them to identify a novel mechanism for ensuring signaling fidelity in bacterial signaling networks. My work concluded that a functional equivalent of eukaryotic subcellular localization is present in prokaryotes to facilitate sensory adaptations in unpredictable and rapidly changing environments.

My research path will integrate important aspects of Molecular, Developmental, and Evolutionary Biology. I believe my interdisciplinary perspective would complement the dynamic research environment at Indiana University, and I am confident that I would be a valuable and productive member of your faculty. Enclosed for your consideration are my curriculum vitae, including my references and publications (with links to reprints) and a statement of my research objectives. A summary of my research achievements and links to my publications can also be found at my website.

I will arrange to have letters of recommendation sent directly to your department. Please feel free to contact me or more references for more information about my previous experience or future plans.

Sincerely,

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Cooperation and Defection: Social Strife in the Staphylococci

Jesse S. Wright

There is constant strife and hardship in the microbial world. Predators lurk at every turn, resources are scarce and unpredictable, and competition for territory can be fierce. Constantly faced with life or death decisions, many prokaryotes have adopted a multicellular lifestyle. A cooperative microbial society can pool efforts to establish formidable defenses, scavenge for nutrients, and explore and acquire new habitats. However, these societies are often vulnerable to cheaters and defectors, which can undermine this process, and reap social benefits at the expense of altruistic cooperators. My postdoctoral studies have focused on the dynamic social behaviors of *Staphylococcus aureus,* a Gram-positive pathogen whose social exchanges appear to display the basic characteristics of cooperation and defection, and represents one of the simplest organisms to encapsulate these features. My research will expand our understanding of the social lives of bacteria, and represents a tractable paradigm for studying group behavior and the evolution of cooperation.

Significance and scope of postdoctoral research

A major transition in our view of bacterial existence in multicellular societies has been the characterization of microbial cell-cell signaling, a process better known as quorum sensing (QS) (Bassler, 2002). In *S. aureus*, QS is mediated by the *agr* operon and controls the regulation of virulence (Novick, 2004). The QS signal is a small autoinducing peptide or AIP, processed from the AgrD propetide by AgrB, and serves as the ligand for AgrC, a membrane-embedded two-component signaling receptor and AgrA, its cytoplamsic signaling partner (Fig. 1). Through the action of an intermediary mRNA effector, RNAIII, *agr* controls the expression ≥150 genes including toxins, superantigens and exoenzymes—major components of the secretome which contribute significantly to pathogenesis. In host tissues, QS triggers the synthesis of the secretome during a rapid growth phase at the onset of infection and is required to counter the host's immune defenses (Wright, 2005b).

The *agr* operon is widely conserved throughout the staphylococci and has undergone a remarkable evolutionary divergence. This has given rise to a unique allele or *agr* type for each species. Intraspecies *agr* divergence has also been identified in several staphylococci including *S. aureus*, which is comprised

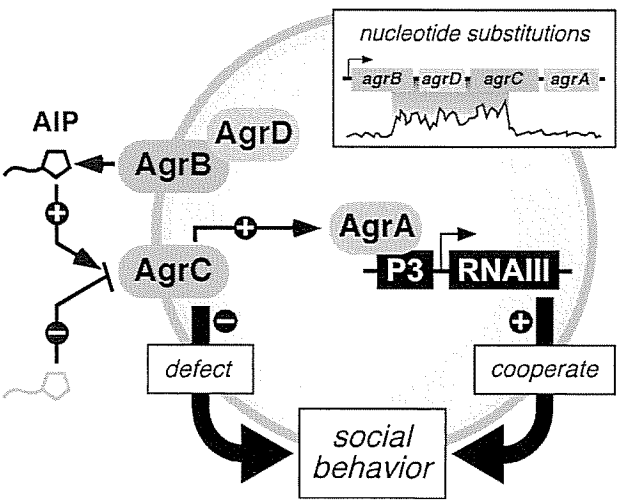

Figure 1. The staphylococcal Agr quorum sensing circuit. The ligand, AIP, processed and secreted by AgrB from AgrD stimulates a phosphorelay signaling cascade through AgrC and AgrA. This activates transcription from the *agr* P3 promoter to drive expression of RNAIII, the primary effector of accessory gene regulation. This response is required to orchestrate virulence and other cooperative social behaviors. Evolutionary divergence at the *agr* locus (inset) has given rise to signaling interference between different *agr* types. Because *agr* controls cooperative behaviors that confer a social benefit, activation and inhibition of the quorum sensing circuit may represent a basic strategy to cooperate or defect with other members of the microbial community.

of four *agr* types. Functionally, each *agr* type deploys a unique AIP for QS signaling. This has generated two consequences for AIP action: If the sender and receiver cells are the same *agr* type, AIPs act as signaling agonists and if the sender and receiver are cells are different *agr* types they act as signaling antagonists (Ji, 1997). Antagonistic AIPs compete for binding to the AgrC receptor and interfere with signaling to block transcription of RNAIII. We discovered that QS interference is based on a universal hydrophobic region found on all AIPs and considerable molecular flexibility in the receptor, AgrC enabling it to accommodate diverse ligands (Wright, 2004). We have also exploited this interference therapeutically in vivo, demonstrating that delivery of an antagonistic AIP prior to *agr* activation in host tissues renders the bacteria vulnerable to the immune system (Wright, 2005b).

Proposed research

Evasion of host defenses is clearly a social benefit conferred by staphylococcal QS. However, we have yet to establish the forces that drive *agr* diversity or the biological significance of QS interference. Staphylococcal interactions outside the host may hold the key to this answer. In contrast to many infectious microbes, pathogenic staphylococci primarily coexist as harmless constituents of the human biota. Roughly a third of the human population is asymptomatically colonized by *S. aureus*—a situation that imposes a significant risk for infection in predisposed subjects, but rarely leads to severe infections in healthy individuals. Very little is known about this staphylococcal lifestyle or the ecological constraints of this existence, but this largely overlooked area may reveal the biological significance of *agr* diversity, since this context is likely to provide ample opportunities for staphylococci to participate in social interactions which result in QS inhibition.

How might group behaviors be influenced by QS in this social context? By applying evolutionary game theory (Maynard Smith, 1974) we may begin to understand the implications of these interactions. If staphylococcal QS confers a species-specific social benefit, akin to cooperation, then QS interference may represent a non-cooperative or defection strategy (Fig. 1). If one views different *agr* types as obligate defectors, then QS interference may represent a cheating mechanism for defectors to benefit from the contributions of cooperators. Alternatively, QS may represent a mechanism for kin recognition (Hamilton, 1964)—that is, being able to distinguish QS signals ensures cooperative behaviors are generated only between genetically similar relatives (i.e, the same *agr* type). In this scenario, QS interference may act as a mechanism used by cooperators to exclude defectors from social benefits.

Using this theoretical framework, my goal is to understand the evolutionary significance and biological consequences of QS interference in the staphylococci. I will first identify QS-generated behaviors and characterize their potential social benefits. Considerable progress has already been made in this area and has set the stage for creating a tractable system to study QS interference. This will be followed by experiments that test the effects of QS inhibition on these social behaviors and address the fates of cooperators and defectors. Related experiments will address the molecular mechanism of QS perception that differentiates AIPs coming from cooperators and defectors. My ultimate objective is to determine the real-world consequences of QS interference in a relevant in vivo model where the effect of QS on the dynamics of colonization and transmission can be evaluated.

Aim I: Characterize the social behaviors of staphylococci mediated by QS. To understand the possible effects of QS inhibition it is first necessary to identify the cooperative behaviors regulated by QS that might benefit the staphylococcal community. We have identified one such behavior—cooperative surface migration—which is regulated by the *agr* locus (Wright, 2006). Superior migration allows cooperative (QS-competent) strains to move into territory and consume resources that are

unavailable to non-cooperative (QS-defective) strains. Moreover, since staphylococci are classically regarded as non-motile the reversal of this notion is an extremely significant finding. To reveal the mechanism of cooperative surface migration, I plan to identify, isolate, and characterize the factor(s) involved. At least one candidate has been identified: δ-hemolysin, a QS-regulated toxin that, notably, is encoded within RNAIII at the *agr* locus. Its intimate link to QS indicates that it is a key factor in mediating cooperative social behavior. Purified δ-hemolysin exhibits surfactant-like characteristics, and suggests that the mechanism of surface migration might be facilitated through the reduction of surface tension. This motile phenotype will be exploited to identify other genes that contribute to surface migration by transposon mutagenesis and targeted knockouts in promising candidates, such as those loci that direct slime production and intercellular adhesion. Also, I will also attempt to identify other possible social behaviors that may also be orchestrated by QS, such as alterations in biofilm architecture, cooperative nutrient acquisition, and altruistic suicide.

Aim II: Investigate the consequences of QS interference on social behaviors. Developmental regulation of sociality during surface migration will be studied using antibiotic markers as well as colorometric and fluorescent reporters (e.g., LacZ, UidA, GFP, and DsRed) to follow the fate of the bacteria and patterns of gene expression. These tools will be applied to other identified social behaviors and will be important reagents to examine the effects of QS interference and the allocation of social benefits. Several social scenarios will be devised to test competing *agr* types on social behavior. These experiments will incorporate important variables such as spatial structure (heterogeneous vs. homogenous) that will also affect the scale of competition (local vs. global). Also, it may be necessary to ectopically reconstruct the four *S. aureus agr* types into the same host background to directly assess the effects of QS during these social situations. This general approach will allow us to ask several important questions: Do obligate defectors (i.e., QS-deficient mutants) gain social benefits at the expense of cooperators? What is the effect on social behaviors when a facultative defector (i.e., a different *agr* type) is encountered by a cooperative group? Do cooperators exclude defectors from social benefits or do defectors benefit at the expense of cooperators? The social outcomes from these exchanges should reveal the evolutionary strategy behind QS interference.

Aim III. Identify the mechanism that triggers QS cooperation and defection. How are QS signals in the staphylococci perceived as cooperative or antagonistic? To identify the AIP binding pocket and unravel the mechanism of signal perception we will utilize the power of bacterial genetics to select for *agrC* mutants with various phenotypes. I have designed a genetic selection to isolate several classes of mutants in AgrC: These include mutations that result in broadened AIP specificity, altered AIP specificity, and constitutive or AIP-independent activity. The ability to obtain receptors with these attributes and the nature and location of the genetic lesions that cause them should be extremely enlightening with regards to the requirements, location and mechanism of cognate AIP recognition. These methodologies will be followed by more extensive biochemical analysis that will incorporate the mutant receptors. This includes using phage display to identify AgrC sequences which may comprise the AIP binding pocket, development of a direct binding assay to measure binding kinetics, and cysteine mutagenesis combined with sulfhydryl labeling to probe the structural rearrangements induced by ligand binding. Finally, I intend to initiate a collaboration to screen a small-molecule library for synthetic QS agonists and antagonists. I have characterized a promiscuous AgrC receptor that is an attractive candidate for in the screen since compounds that activate this mutant are likely to represent possible inhibitors of the native receptors (Wright, 2004). Compounds identified by this screen will serve as important reagents to further probe the molecular requirements for AIP-AgrC recognition, will be incorporated into my social behavior studies and stand to represent promising candidates for anti-staphylococcal therapy.

Aim IV: Investigate the possible role of QS cooperation and defection in species diversity. It is very likely that cooperation and defection will have some effect on the allocation of social benefits. One of my long-term objectives is to determine the biological consequences of these interactions on staphylococcal evolution. There is a strong link between *agr* type and genetic relatedness in *S. aureus*— and, across the species, *agr* variation shows strong phylogenetic congruence with 16S rRNA (Wright, 2005b). Could QS interference in *S. aureus* possibly be involved in incipient speciation? One possibility could be that social behaviors driven by QS cooperation and defection are driving ecological isolation and constraining horizontal gene flow. This may be difficult to test if I do not identify any hints of *agr*-dependent segregation or displacement in our social behavior studies. However, I will attempt to investigate this possibility by examining the role of QS on colonization and transmission in a relevant in vivo model. I have initiated development of a promising colonization model using *Drosophila melanogaster*. High numbers of staphylococci can be harvested from individual flies for >1 week suggesting this model may be robust enough to support this type of analysis. It may also provide a novel approach to identify genes involved in this non-pathogenic, commensal lifestyle. I intend to continue development of this model to ultimately determine the possible role of QS on the dynamics of staphylococcal colonization and transmission.

Perspectives

Faced with a dwindling pipeline of new antibiotics and an alarming resurgence in antibiotic resistance, understanding the social lives of bacteria may generate more productive methods to control infectious diseases. In particular, such comprehension stands to reveal fundamental aspects of bacterial physiology and behavior that are important for competition in the complex biosphere. Expanding our knowledge of these strategies has the potential to reveal novel approaches for controlling infectious microbes, which may circumvent the selective pressures that generate resistance. The details of staphylococcal QS could also uncover important insights into the molecular mechanisms of receptor activation and inhibition, which could have broad implications for rational drug design and discovery. In addition, studies addressing the biological rationale of staphylococcal social behaviors will lend a unique perspective to our view of biological altruism, as well as to the role of cooperation and defection in the process of speciation, illuminating the evolutionary origins of multicellular life.

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