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Dear Search Committee,

I am writing to apply for the position of Assistant Professor at Indiana University in Systems Microbiology that was recently advertised.

I am currently an Assistant Professor in the School of Biology at the Georgia Institute of Technology. My lab is up and running after receiving a grant from NIH for the study of chemosensory regulation of gene expression in *Myxococcus xanthus*. We are employing various techniques including genetics, molecular biology, biochemistry, genomics, and bioinformatics to assess the role and integration of multiple paralogous chemosensory systems that regulate the complex multicellular life cycle of *M. xanthus*. In addition, we are investigating the mechanism of chemotaxis in *Bacillus subtilis* as a paradigm for chemosensory signal transduction. A brief outline of these projects is included.

Thank you for considering my application.

Sincerely,

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# Kirby Lab

## Chemosensory Signal Transduction in Bacteria

The major research efforts in my laboratory broadly focus on chemosensory signal transduction in Bacteria. One of our primary interests is to elucidate the role of multiple paralogous chemosensory systems that regulate motility and gene expression during development in *Myxococcus xanthus*. A second major project focuses on the characterization of the mechanism of chemotaxis in *Bacillus subtilis*. A brief description of these projects is given below.

### I. Chemosensory regulation of motility and gene expression in *Myxococcus xanthus.*

*Myxococcus xanthus* displays a complex life cycle and has been a model organism for studies in intercellular communication, multicellular development, and biofilm formation. During vegetative growth, cells exhibit coordinated behavior to feed on colonies of other bacteria or to spread on nutrient agar plates (Fig. 1). Cells utilize both Type IV pili and an unknown motor thought to depend on polysaccharide hydration and extrusion to regulate gliding motility on solid surfaces. Importantly, both motility systems are required for completion of the multicellular developmental program. When starved, cells coordinate their motility to form aggregates which later mature into spore-producing fruiting bodies. Control of motility and regulation of gene expression during fruiting body formation is known to depend on intercellular signaling. The various signals include A-signal (amino acids), C-signal (a cell surface associated protein), dilauroyl phosphatidylethanolamine derivatives, and amine polysaccharides. Several putative receptor proteins including SasS, DifA, and FrzCD are also required for fruiting body formation. However, a direct link between the known signals, the known signal transduction pathways, and control of motility has not yet been made.

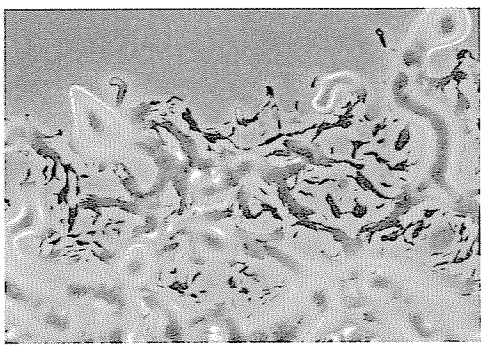

**Fig. 1. Swarming pattern of wild-type *M. xanthus* cells on a solid surface.** Cells were grown on rich media agar plates. The photomicrograph (200x) was taken after 5 days growth at 32 C.

Because extracellular signals are known to affect motility via chemosensory signal transduction pathways, I hypothesized that chemotaxis receptors (MCPs) would be involved in the regulation of the complex motility displayed during fruiting body formation in *M. xanthus*. Using standard molecular biology and biochemical techniques, I was able to identify several *mcp* homologs located within several previously uncharacterized *che* clusters (performed during my postdoc in the Zusman lab). Acquisition of the *M. xanthus* genome has now confirmed that there are 8 *che* loci and 21 *mcp* receptor genes on the 9.2 Mb genome. While this number of receptors is average for prokaryotic genomes, 8 *che* clusters is the largest number known to date. The role of each of the Che systems and their potential integration within the signal transduction network during development has become the major focus of our research.

**The major biological question is what do each of the multiple *che* loci control?** If all Che systems control motility, how are those systems designed to create "molecular insulation" such that cross talk is not significant? If cross regulation does occur, what is the mechanism of integration for the signaling network? If each chemosensory system functions independently, what are the signals and outputs? In order to address these questions, we are analyzing the previously uncharacterized Che systems (all but Dif and Frz) using genetic, biochemical, molecular, genomic, and bioinformatic techniques.

Analysis of the Che3 system led to the surprising discovery that this chemotaxis-like signal transduction system affects developmentally regulated gene expression without affecting motility or chemotaxis. Although the Che3

system comprises homologs to CheA, CheB, CheR, CheW, and two MCPs, the output of the system is unusual with respect to the prototype found in *E. coli* for control of motility (CheY). Instead, the Che3 system feeds through CrdA, a homolog of the NtrC family of $\sigma^{54}$-dependent transcriptional activators (Fig. 2). This was the first example of a chemotaxis-like system that directly affects gene expression and is dedicated to that function rather than to the control of motility. Importantly, several recent examples of chemotaxis systems in other species including *Rhodospirillum centenum* and *Pseudomonas aeruginosa* suggest that chemosensory regulation of gene expression may occur widely in bacteria and is therefore a very exciting avenue of investigation.

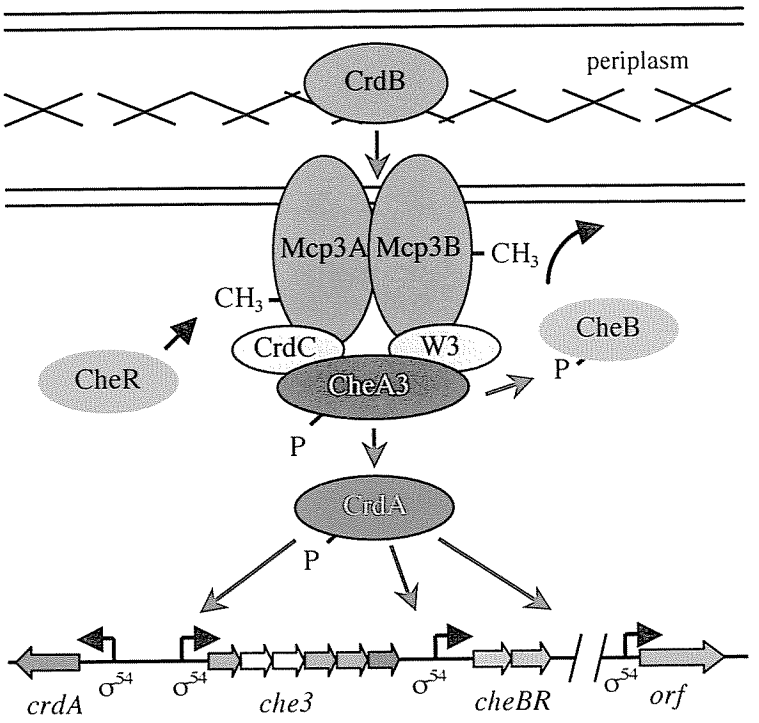


**Fig. 2. Model for Che3 regulation of gene expression.** Proteins are color coded to match the corresponding genes shown below. The *che3* locus is comprised by *crdB*, *cheW3*, *crdC*, *mcp3A*, *mcp3B*, and *cheA3* (left to right). *cheB* and *cheR* are expressed from a different promoter. Other genes (*orf*) are thought to be regulated directly by CrdA.

**The project to characterize the Che3 signal transduction pathway is currently funded by the NIH.** Our AIMS are to: **1)** characterize the phosphorelay component of the signaling pathway, **2)** characterize both the periplasmic and cytoplasmic components of the receptor signaling complex, **3)** analyze the role of methylation-dependent adaptation, and **4)** identify potential outputs controlled by the Che3 system.

We hypothesize that damage to the outer membrane is sensed by the Che3 system and leads to expression of genes that control sporulation. Damage to the outer membrane is predicted to affect CrdB which shows high similarity to lipoprotein receptors. CrdB would then interact with the Mcp3A-Mcp3B receptor complex and induce a conformational change transmitting information to CheA3. The flux of phosphoryl group transfer from CheA3 to CrdA would be altered in a manner similar to that observed during chemotaxis. Because several mutants in the Che3 system lead to increased expression of developmentally regulated genes even under vegetative conditions, the Che3 signal transduction system is thought to repress gene expression during vegetative growth. Recent evidence suggests that gene expression is enhanced during development indicating that the Che3 system is capable of tuning gene expression either up or down depending on the status of the developmental program. Our evidence suggests that CheA3 acts as a phosphatase during vegetative growth to repress expression and as a kinase during development to enhance expression. We hypothesize that Mcp3A and Mcp3B play a role in differential regulation of CheA3 kinase-phosphatase activity and that CheW3 and CrdC are partitioned between the receptors. Additional results indicate that methylation of the receptor complex affects developmental gene expression. We also have evidence indicating that *crdA* expression is autoregulated and controls expression of the divergently transcribed *che3* cluster.

The findings from our analysis of the Che3 system have led us to investigate the remaining uncharacterized systems, Che5, Che6, Che7, and Che8. Each of the *M. xanthus* Che systems encodes a single CheA protein as well as homologs to the other chemotaxis components. For any given chemosensory system, CheA is the

central processor through which all information passes. Therefore we have mutated each *cheA* gene in the wild-type background. **Mutants in each of the 8 *cheA* homologs give distinct phenotypes** suggesting that each kinase defines a specific signal transduction pathway with a specific output. This data also strongly suggests that each system functions with a level of molecular insulation and does not display significant crosstalk. Our preliminary results indicate that Che6 affects C-signal dependent aggregation, Che7 affects Type IV pilus based motility (Fig. 3), and Che8 affects control of riboflavin biosynthesis by an unknown mechanism. As is the case for the *che3* cluster (Fig. 2), each of the other 7 *che* clusters contain non-chemotaxis genes. We hypothesize that those non-chemotaxis genes encode the critical proteins that interact with the chemosensory systems to regulate functions different from motility. Studies are underway to investigate the unique aspects of each of these paralogous chemosensory systems.

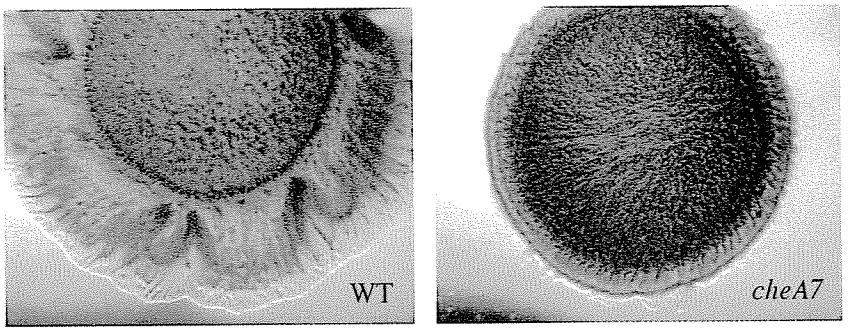


**Fig. 3. The *cheA7* mutant displays reduced pilus based motility.** Cells were grown to mid log phase and spotted on 0.3% agar plates containing rich media. Photographs were taken after 2 days at 10x magnification. Motility on 0.3% agar surfaces is thought to depend on Type IV pilus based motility. The wild type is shown in the left panel and the *cheA7* mutant is shown at right.

## II. Mechanism of Signal Transduction in *Bacillus subtilis*

The second important area of research in my laboratory focuses on the study of chemotaxis in *B. subtilis*. My interest in this subject has continued since my PhD training in the Ordal lab at the University of Illinois. I published several papers on various aspects of the chemosensory mechanism that revealed deviations from the paradigm in *E. coli*. Some of that work led to further analyses that have revealed even greater differences between the mechanisms found to control chemotaxis in *E. coli* and *B. subtilis*. Importantly, extensive analysis of prokaryotic genomes allows us to extend our understanding of those differences across the Bacterial and Archaeal domains.

The combination of genomic analyses of chemotactic prokaryotes (Zhulin lab) and biochemical and genetic evidence generated from studies on both *B. subtilis* (Ordal lab) and *Halobacterium salinarum* (Spudich lab) indicate that ***B. subtilis* should be considered the new paradigm for the study of chemotactic signal transduction**. *B. subtilis* has been shown to utilize CheC (a phosphatase) and CheD (a deamidase) to regulate chemotaxis. While the majority of prokaryotes possess homologs to *cheC* and *cheD*, *E. coli* does not. Additionally, the chemoreceptors are differentially methylated in *B. subtilis* and *H. salinarum*, but not in *E. coli*. Therefore, analysis of the mechanism of chemotaxis in *B. subtilis* where CheC and CheD have been most thoroughly studied is important for our understanding of chemosensory signal transduction in all prokaryotes.

Although much is known about chemotaxis in *B. subtilis*, many aspects of the signal transduction pathway remain unknown. We are investigating the following specific areas:

1) CheD is required for signal transduction through McpC but not for signal transduction through McpB. This difference indicates that there are at least two distinct signal transduction mechanisms in *B. subtilis*. A domain found in most chemoreceptors known as the HAMP domain is present in McpC and is required for CheD-dependent signaling. However, McpB also possesses a HAMP domain but does not require CheD to transduce signals. Therefore we hypothesize that there are functional differences between the HAMP domains of McpB

and McpC. Genomic analysis of the family of HAMP domains and CheD proteins is underway to identify specific residues that display covariance between the two putative types of HAMP domains and CheD homologs (Zhulin lab). That analysis will allow us to target specific residues for mutation and should greatly enhance our studies of the role of CheD and its targets for modification.

2) CheC was very recently shown to act as a phosphatase on the response regulator CheY. CheC and CheD are also thought to interact in a 1:1 stoichiometric ratio and interact with the receptor complex. Because CheY-P diffuses from the receptor complex to the flagellar switch complex, subcellular localization of the CheC phosphatase plays a critical role in its function. Further analysis of CheC phosphatase activity, its regulation by other Che proteins, and CheC localization will be analyzed.

3) Positive identification of the specific sites of methylation are still unknown for all organisms other than *E. coli*. Genomic analysis of nearly all prokaryotic chemoreceptors in the database suggests that the MCPs in *B. subtilis* should be considered as prototypical (Zhulin lab). Because differential methylation changes have been shown to occur for *B. subtilis* McpB, further analysis of the regulation and role of these methylation changes is necessary. Biochemical characterization of the sites of methylation is the top priority in this area.

**Summary**

Our analysis of the multiple paralogous chemosensory systems in *M. xanthus* will lead to a better understanding of the complexity of two-component signaling and regulation of gene expression. We are gaining insights into chemosensory regulation of $\sigma^{54}$-dependent transcription, regulation of CheA kinase/phosphatase activities, methylation-dependent regulation of gene expression, control of pilus-based motility, and prokaryotic development. Analysis of the mechanism of signal transduction during chemotaxis in *B. subtilis* will lead to greater knowledge of the roles of CheC and CheD during chemosensory signal transduction in all prokaryotes. Comparative analyses between the paralogous chemosensory systems of *M. xanthus* and the paradigm system in *B. subtilis* gives us a unique perspective on chemosensory signal transduction in prokaryotes.

## TEACHING INTERESTS

Throughout my academic career, I have had excellent experiences both as a student and as a teacher. I consider teaching in the classroom and laboratory settings to be equally important. I hold in high regard those who inspired me over the years. I have attempted to follow their lead and have received some recognition for my efforts.

As a student at Illinois, I had the opportunity to be a teaching assistant in advanced general chemistry with Professor Steve Zumdahl where I learned a great deal about the art of communication in science. During my PhD thesis work in Biochemistry, I learned much from Professor George Ordal about creating a supportive atmosphere for students to flourish. I try to continue that practice in my laboratory today.

As an assistant professor at Georgia Tech, I have taught advanced microbial physiology, a microbiology "project" laboratory course for upper level students, introductory microbiology, and a section of "foundations" in molecular biology for graduate students.

Because of these experiences and my research interests, I would enjoy teaching courses ranging from microbial physiology to prokaryotic development. I believe that I can make a significant contribution to your faculty as a teacher in both laboratory and classroom settings.