UW College of Agriculture and Natural Resources

Global Perspectives Grant Program

Project title: Outer membrane fusion and protein biogenesis in myxobacteria

Award period: Spring 2015

Principle Investigator: Daniel Wall

Department: Molecular Biology

Email: dwall2@uwyo.edu

Amount spent: $2,94.73

Non-technical summary: Bacteria, like humans, can recognize their kin and participate in cooperative behaviors. The benefit of social interactions can in turn exceed the abilities of the individual and can improve the fitness of the population. Myxobacteria are a model organism for studying kin interactions because they elicit many complex social behaviors and there are advanced genetic tools to study them. One such behavior is the ability of thousands of cells to coalesce and build multicellular fruiting bodies, in which growing cells differentiate into spores in response to starvation. Spores are dormant cells, analogous to a plant seed, which can exist for many years without nutrients and are resistant to stresses, such as dehydration, temperature shifts and more. Once food becomes available spores germinate and the cells again grow. In my laboratory we study how myxobacteria recognize their kin and here we have discovered a cell surface receptor called TraA that mediates these interactions. TraA is interesting for this reason and because it acts as a fusagen for the transient fusion of the outer membrane between cells. Once membrane fusion occurs the then cells exchange hundreds of different proteins and lipid molecules. This exchange of cell content then leads to social behavior changes. Because TraA has unique and important roles for cells we want to understand how it works. In this regard I conducted a sabbatical in Dr. Trevor Lithgow's lab at Monash University, Melbourne, Australia, to gain expertise in working on outer membrane protein assembly as it pertains to TraA function.

REPORT**:** From January 5 to June 12, 2015, I conducted a sabbatical in Dr. Trevor Lithgow's laboratory in the Microbiology Department at Monash University (pictured below). The Lithgow laboratory conducts pioneering research on how proteins are assembled in the outer membranes of Gram-negative bacteria. Outer membrane or cell surface proteins are particularly important because they interact with the environment and with other cells. For example, in pathogenic bacteria cell surface proteins interact with host cells for virulence functions and they are the proteins detected by the immune system to which a response is mounted. In myxobacteria, cell surface proteins govern kin recognition and social interactions between cells. While visiting the Lithgow lab I interacted with his large group, about 15 scientists, and learned about their cutting edge research into outer membrane protein assembly. My initial plans to study TraA/B in *Myxococcus xanthus* were partly hampered, because of complexities (I was unaware of) associated with the Australian's government quarantine polices on shipping 'new species' into the country. Nevertheless I did conduct experiments of TraA and TraB in a heterologous host (*Escherichia coli*). From these studies I gained insights to their localization in cells and how to best approach questions about TraA/B protein assembly/function in *M. xanthus*. In addition, to disseminating

information about the University of Wyoming and our research to the Lithgow lab, on May 18, 2015, I gave an invited seminar to the Microbiology Department at Monash University, which was advertised campus wide (total enrollment 65,000 students; 25,000 on the Clayton campus).

One of the unusual properties of the TraA protein is that it contains a large number (79) of cysteine residues. Cysteines are unique among the 20 naturally occurring amino acids found in proteins, because their side chains can form covalent (disulfide) bonds between cysteine residues in an oxidizing environment, e.g. a protein on the cell surface. Thus, unlike the standard primary structure of a protein, where amino acids are simply linked together like beads on a string, disulfide bonds cross-link particular cysteine residues and change the linear primary structure into a mesh or network of covalent bonds in the protein, which in turn has profound implications on protein structure and function. Because TraA contains so many cysteine residues it implies that these amino acids play an important role in TraA functional. To gain insight into how cysteine residues function in TraA and how to best experimentally address this complex issue I met with a leading world authority on cysteines and disulfide function. This individual is Professor Philip Hogg at the University of New South Wales, Sydney Australia (picture below). Dr. Hogg gratuitously hosted me for a visit and he provided valuable suggestions for experimental approaches to test functional roles of cysteines in TraA function.

Future plans: From this sabbatical I was developed rapports with Drs. Lithgow and Hogg. From these interactions there are plans to interact on future research, at scientific meetings and scientific publications. Hopefully, one or both of these researchers will one day be able to visit the University of Wyoming.

Impacts to the College/University/State: The primary impact of this Global Perspective grant is that it has strengthen the research program of the PI. This has been achieved by learning new techniques, insights and developing new collaborations. In turn future grant proposals by the PI will be strengthen with the hope that significant federal research dollars will continue to support the lab and undergraduate and graduate students that conduct research here at UW.

Biological Sciences building that houses the Microbiology Department, Clayton campus, Melbourne, Australia.

Lowy Cancer Research Centre at the University of New South Wales, Sydney Australia, which houses Dr. Philip Hogg's laboratory.