J.T. Beatty, Dept of Microbiology & Immunology, University of British Columbia, Vancouver, Canada.

Project Descriptions

1. **Study of the regulation and expression of novel Gene Transfer Agents** is a major research focus. These evolutionarily crucial agents of horizontal gene transfer can mediate genetic exchange between species, unlike the conventional vertical, parent to offspring, transmission.
We hypothesize that, in the model system of a gene transfer agent (GTA) produced by the bacterium Rhodobacter capsulatus, cellular differentiation is controlled by a small regulatory RNA (sRNA) and a signaling phosphorelay controlled by the sensor kinase protein CckA. The CckA protein alternates between kinase and phosphatase activities in response to changes in environmental signals. Our overall research has three main goals:

- Determine how an sRNA functions in R. capsulatus differentiation.
- Elucidate the function of CckA phosphorylation (kinase) and dephosphorylation (phosphatase) activities related to GTA production and the acquisition of GTA-borne genes.
- Determine the interplay between the sRNA and phosphorelay regulons.

The student will focus on the CckA protein. An E. coli expression system will be used to overexpress soluble wild type R. capsulatus CckA protein (which is normally membrane-bound), by transforming E. coli with a plasmid encoding the cytoplasmic (soluble) segment of CckA (a plasmid encoding His-tagged protein is already available). The protein would then be isolated and purified to be used in a PhosTag-containing SDS-PAGE assay to measure protein phosphorylation. If wild type CckA is able to autophosphorylate when incubated with ATP, as would be identified in the SDS-PAGE assay, then the same procedure would be used to test if the mutant CckAs are still able to display autophosphorylation to the same extent after incubation with ATP.
This position is a balance of essential lab maintenance tasks, and direct participation in a research project. Preparation of growth media, stock solutions of all molecular biology chemical solutions, and cleaning glassware are essential duties of this position. The student must also have experience in cultivation of microbes (aseptic technique, streaking agar plates, etc.) as well as some hands-on experience in DNA and or protein manipulation. For example: plasmid purification, agarose gel electrophoresis of DNA samples, PCR, gene cloning; and/or SDSPAGE, protein purification, column chromatography, assays of enzyme activity.
On the first day of work, the student will complete (or confirm completion of) an on-line UBC safety and orientation course. Once certified, the student will receive a thorough laboratory orientation, given by the laboratory manager. All safety issues and lab specific polices and protocols will be clearly explained. All material covered in this orientation will be available in written form.
With completion of the tasks assigned to this lab assistant position, the student will be given the opportunity to conduct a research project. Mentoring with the principal investigator and lab manager, the student will learn to prepare bacterial cultures, use microscopic and biological assays to perform morphological, and physiological examination of these cultures. The student will be taught methods of spectrophotometry, enzyme assays, growth studies, and gene transfer agent assays.
The student will be asked to present their research in English at a bi-weekly lab meeting. The ability to convey their work in a clear and understandable manner is of paramount importance. Such presentation practice improves indispensable communication skills. Lab members are eager and enthusiastic in their support in these meetings. When attending presentations, the student will be strongly encouraged to engage in constructive criticism, and questioning. The relaxed and friendly group meeting atmosphere is highly conducive to open questioning, debate, and discussion.

2. M**icrobes that capture solar light and convert its energy to electrical current**, using special proteins and pigments combined in a biological photosystem or photosynthetic reaction centre (RC). My approach ranges from finding new species to engineering proteins with new biophysical properties. The use of biological photosystems in photovoltaics has become possible due to our improved knowledge of these systems and new gene engineering methods, but we still need to answer many questions about the design and creation of photon and electrical circuits in proteins, to make full use of the potential of these systems.
The application of biological photosystems to photovoltaics has become feasible as a result of: gene engineering methods; the solution of the 3-D structures of photosystem membrane protein complexes; and the quantitative understanding of the primary electron transfer processes of photosynthesis. The reaction center (RC) of the bacterium Rhodobacter sphaeroides is the simplest and perhaps the most easily-studied photosynthetic complex, with a ~200-fold longer recombination time of separated charges than in silicon-based devices. We have made major advances in the study of the R. sphaeroides and related RCs, but such RCs are not able to withstand high temperatures that might be encountered in solar energy applications, such as on a rooftop. However, the bacterium Chloroflexus aurantiacus grows at temperatures of 65-70º C. Thermophiles such as C. aurantiacus lack genetic malleability and are difficult to cultivate, and so we will use heterologous expression to circumvent these obstacles. The protein-encoding genes from C. aurantiacus will be expressed in our system using an R. sphaeroides strain that lacks its own RC genes, while providing chlorophylls and other cofactors. In preliminary experiments we created

synthetic versions of the C. aurantiacus RC genes and found that a functional RC was assembled in vivo, which could be purified for in vitro applications. We wish to move onward from this promising result to improve the stability and yield of this RC.
This position is a balance of essential lab maintenance tasks, and direct participation in a research project. Preparation of growth media, stock solutions of all molecular biology chemical solutions, and cleaning glassware are essential duties of this position. The student must also have experience in cultivation of microbes (aseptic technique, streaking agar plates, etc.) as well as some hands-on experience in DNA and or protein manipulation. For example: plasmid purification, agarose gel electrophoresis of DNA samples, PCR, gene cloning; and/or SDSPAGE, protein purification, column chromatography, assays of enzyme activity.
On the first day of work, the student will complete (or confirm completion of) an on-line UBC safety and orientation course. Once certified, the student will receive a thorough laboratory orientation, given by the laboratory manager. All safety issues and lab specific polices and protocols will be clearly explained. All material covered in this orientation will be available in written form.

With completion of the tasks assigned to this lab assistant position, the student will be given the opportunity to conduct a research project. Mentoring with the principal investigator and lab manager, the student will learn to prepare bacterial cultures, use microscopic and biological assays to perform morphological, and physiological examination of these cultures. The student will be taught methods of spectrophotometry, enzyme assays, growth studies, and gene transfer agent assays.

The student will be asked to present their research in English at a bi-weekly lab meeting. The ability to convey their work in a clear and understandable manner is of paramount importance. Such presentation practice improves indispensable communication skills. Lab members are eager and enthusiastic in their support in these meetings. When attending presentations, the student will be strongly encouraged to engage in constructive criticism, and questioning. The relaxed and friendly group meeting atmosphere is highly conducive to open questioning, debate, and discussion.