



# Department of Microbiology

## School of Biomedical Sciences

### 2010 Honours Programs in Microbiology

<b>2010 Honours Coordinator</b>	
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## 2010 Honours Programs in Microbiology

The Honours programs for both BMS and BSc contain coursework and an independent research project. The objectives of these courses are to develop the laboratory skills required for research in microbiology and the ability to evaluate critically microbiological research. Students also achieve a detailed understanding of specialised topics in microbiology and enhance their communication skills in written and oral presentations.

The Department looks forward to welcoming you in 2010. We feel that our friendly, constructive and highly productive working environment provide an excellent opportunity for honours students to develop an understanding of the research process and to achieve their full research potential.

### Formal Application Process

Application for Microbiology Honours entry involves a two part application process.

1. Formal application to the relevant faculty by November 20.

B. Sc (Hons): <http://www.sci.monash.edu.au/staff/honours.html>

B. Biomed. Sci (Hons): <http://www.med.monash.edu.au/biomed/honours/>

2. Submission of project preferences to Prof Julian Rood (no later than November 23, 2008).

### Research Projects

The research project is the major component of both programs. All efforts are made to accommodate students in the laboratory of their choice, and to develop research projects that take into account the student's, as well as the supervisor's interests. Brief outlines of the available projects for 2010 are in the following section.

### Supervisor Interviews

Applicants are encouraged to discuss research projects with potential supervisors at any suitable time, by appointment. Following these discussions, students will need to give Prof Julian Rood their Microbiology application forms (see last page) indicating their project preferences, and any additional documentation required. You do not need to wait until November 23 to hand in your preference forms, the earlier the better.

### Projects outside the Department

It is possible for students to complete their course work within the Department of Microbiology at Clayton, and their research project off-campus. Under these circumstances, students must travel between locations when required. The thesis examination takes place at the same time for all students enrolled through Microbiology.

### Microbiology coursework

The course work conducted within the Department of Microbiology consists of short courses termed colloquia. BSc students need to complete two colloquia, which together

comprise the unit MIC4200: Advanced Studies in Microbiology, and BMS students one colloquium in addition to a common core coursework component (see below). Each colloquium is held during a one month period in the first half of the year, so that the course work is usually completed, and students receive some feedback on their progress, by mid-year. The formats of the colloquia vary. Most involve reading recent research papers, an oral presentation, and a written assignment.

### **BMS common core coursework**

In addition to one colloquium, all BMS Honours students must complete a centrally assessed common coursework component consisting of:

- A statistics module, an accompanying workshop and test
- A written critique of a scientific paper, in a three-hour examination format

### **Literature survey**

During first semester the students must submit a literature survey on their research project. The literature survey (which can be used as the basis for the introduction in the final report) allows the identification early in the year of those students who have problems with English expression. It also, of course, compels the students to become thoroughly conversant with their area of research.

### **Additional requirements**

The programs will commence on February 22, 2010 with a series of introductory lectures, before the students start work on their research projects. These lectures contain information on the course, departmental facilities and laboratory safety. In the second half of the year students may be given specific training in the presentation of written reports, and in oral presentation of their work. It is compulsory for students to attend the introductory lecture course, all departmental seminars, and any short courses on written and oral presentations

### **Assessment**

Final assessment of the BSc Honours program follows the format:

Literature survey	7.5%
Research report/report review	60%
Seminar	7.5%
Colloquia (2 x 12.5%)	25%

Final assessment of the BMS Honours program follows the format:

Literature survey	5%
Research report/report review	60%
Seminar	10%
Colloquia (1 x 10%)	10%
Statistics Module	7.5%
Common written component	7.5%

## Eligibility

### **Monash BSc Students**

Entry to the course is restricted to those students who have qualified for the BSc (all subjects completed before enrolment), and have an average of at least 70% in 24 points of relevant level-three science units. This generally includes at least 18 points of Microbiology units. Under special circumstances students who have a high credit average in Microbiology **may** be admitted, provided that they have also obtained an average of at least 65% in their remaining level-three units. Alternatively, such students may be permitted to enrol in a Master of Biomedical Science (Part1) program. Students studying combined Science degrees must be eligible for the award of BSc.

### **BSc Graduates of Other Universities**

As for Monash students, applicants are required to have a BSc and distinction grades in Microbiology or closely related subjects. **A certified copy of the applicant's academic record and a statement to the effect that they have qualified for a pass degree are required as soon as they are available.**

### **Monash BMS students**

Students must have completed all requirements for the award of the pass degree of Bachelor of Biomedical Science offered at the Clayton campus. They must also have an average of 70% or higher in at least 24 points at third year level, with 12 points from third year core units. Heads of Departments may make a case for students with a grade average in the range of 65 to 69% who have demonstrated research potential.

### **BMS graduates from other universities**

Students applying for admission based on a qualification other than the pass degree of Bachelor of Biomedical Science offered at the Clayton campus will need to demonstrate that they have achieved an appropriate standard in studies comparable to 24 points of BMS subjects as stipulated above.

## Part-Time Study and Mid-Year Entry

The department prefers students to study on a full-time basis. However, it may be possible under special circumstance to complete the Honours degree in two consecutive years by doing the course work and research work in separate years. It may also be possible to start the course mid-year. In both of these circumstances, the arrangements are made on an individual basis between applicants and supervisors.

## Research Projects 2010

Dr Michelle Dunstone

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### Structural Biology, Protein biophysics

#### **Molecular hole punchers: from immunity to bacterial disease**

**(Dr Michelle Dunstone, Dr Tamas Hatfaludi and Prof James Whisstock, Dept of Biochemistry)**

Pore Forming Toxins (PFTs) are proteins commonly identified as bacterial virulence factors, proteins of the immune system and animal venoms. These molecules possess the ability to change shape from water soluble single proteins to lipid membrane inserted ring-shapes consisting of 12 – 50 molecules. These ring-shapes act as pores in the target cell membrane that can result in death of the cell by lysis or delivery of other toxins. This research focuses on the function of a recently united CDC/MACPF superfamily of pore forming toxins. This research aims to determine the pore structure of pore forming toxins. Comparison of the structures before and after pore formation will provide insight into the mechanism of function of all MACPF/CDC pore forming toxins in disease and immunity. Lab skills taught include molecular biology, protein biochemistry, cell/liposome lysis assays, biophysical techniques, electron microscopy and X-ray crystallography.

#### **The role of methionine-binding proteins in the survival of bacteria *in vivo*** **(Dr Michelle Dunstone and Dr Tamas Hatfaludi, Dept of Biochemistry)**

*Pasteurella multocida* is a Gram-negative pathogen that is able to cause disease in a wide range of hosts, including fowl cholera in birds, atrophic rhinitis in pigs, haemorrhagic septicaemia in cattle, snuffles in rabbits and, more rarely, wound abscesses and meningitis in human. Despite several identified virulence factors, the mechanisms by which *P. multocida* can survive in the environment and successfully infect and cause disease in various hosts remains unclear. We have found that the methionine binding MetQ homologue, PlpB plays a vital role in the growth of *P. multocida* *in vivo*. These findings illustrate for the first time the biological significance of a methionine-binding protein in a physiological environment, a finding probably applicable to all bacterial species. This project aims to determine the structure of the methionine binding proteins and identify the specificity of the methionine binding. Techniques used will include, molecular biology, site directed-mutagenesis, protein-binding assays, protein chemistry, virulence studies in animal model, crystallisation and X-ray crystallographic studies.

#### **Structural Studies of the “Burger Bug”**

**(Dr Michelle Dunstone, Dr Tamas Hatfaludi, Dept of Biochemistry in collaboration with Dr Liz Hartland, University of Melbourne).**

Enteropathogenic *Escherichia coli* (EPEC) is a pathogenic form of *E. coli* that cause severe gastroenteritis particularly in children and immunocompromised people. Outbreaks

of EPEC are synonymous with contaminated beef products such as undercooked beef burgers (the “burger bug”). EPEC attaches to the cells of the intestinal epithelium. EPEC uses the Type Three Secretion System (TTSS) to deliver many proteins, known as effectors, into the host cell. These effectors are used by EPEC to subvert cellular processes that are used by the pathogen to benefit pathogen replication and contribute to disease. However, little is known about how these effectors function. This project will aim to identify the mechanism of function of key effector proteins using X-ray crystallography in conjunction with cell biology techniques. Lab skills taught include bioinformatics, molecular biology, protein chemistry, X-ray crystallography, protein-protein interaction assays, cell biology and microscopy.

## **A/Prof Hans J. Netter**

Two vacancies

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### **Modified HBV VLPs as vaccination tools against infectious diseases. (A/Prof Hans Netter & Dr Wan-Shoo Cheong)**

Virus-like particles (VLPs) are used as vaccines for the prevention of infections against hepatitis B virus (HBV) and human papilloma virus (HPV). VLPs represent an effective vaccine modality as they are highly immunogenic due to their spatial and repeated sub-unit structure providing epitopes in several copies on a defined particle. The small hepatitis B surface (envelope) antigen (HBsAg) has the capacity to self-assemble with host-derived lipids into empty spheres of 22nm in diameter. HBsAg VLPs are the sole antigenic component of one of the most successful vaccines (hepatitis B). Clinical trials have also shown that they are highly successful delivery systems for foreign epitopes and protein domains. The ability of VLPs to serve as carriers of B cell and CTL epitopes derived from either the parental virus or foreign sources has further enhanced and broadened their potential as prophylactic and therapeutic vaccines.

Research in my laboratory focuses on the design of chimeric VLPs with the capability to induce protective immune responses, and to learn about the mode of their action and the involved processing pathways. We have engineered chimeric HBsAg VLPs by introducing sequences or vaccine targets from hepatitis C virus (HCV), human immunodeficiency virus (HIV-1) and influenza virus, and proven that both humoral and cellular immune responses can be induced which are specific for the inserted foreign epitopes. Projects are available for the development of VLPs composed of HBsAg proteins fused to protein domains derived from pathogens with unmet medical need, and to determine the quality of the immune response at the level of the innate and adaptive immune system. As VLPs resemble native viral structures, the outcomes have also direct implications for virus-immune system interactions.

### **Requirements essential for VLP formation, stability and secretion competence including the capacity to facilitate assembly of the satellite virus hepatitis delta. (A/Prof Hans Netter & Dr Wan-Shoo Cheong)**

Hepatitis delta virus (HDV) is a satellite virus and depends on the presence of HBV to provide the HBsAg envelope protein for viral assembly and secretion. HDV remains today

the only recognized transmissible agent of its type in the entire animal kingdom. To gain a detailed understanding of the HBsAg and HDV secretion process, we investigate HBsAg mutant proteins regarding their ability to form VLPs, to facilitate HDV assembly and determine their capability to form secretion-competent HDV particles. This will lead to a profound understanding of the secretion process of HBV and HDV.

Projects are available i) with the focus on chimeric HBsAg/HIV or HBsAg/influenza VLPs and in the assessment of their immunogenicity with regard to CTL- and B-cell immune responses and ii) to study the interaction of HBV and HDV.

## **Prof Julian Rood**

Two vacancies

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### **Functional Biology of Bacterial Pathogens**

#### **Regulation of extracellular toxin production in *Clostridium perfringens* (Dr Jackie Cheung & Prof Julian Rood)**

The causative agent of gas gangrene, *Clostridium perfringens*, elaborates many extracellular toxins and enzymes during exponential growth. Of these toxins,  $\alpha$ -toxin and perfringolysin O have been implicated in the disease process. Studies in this laboratory have shown that the production of these toxins is regulated by the VirS/R two-component signal transduction system. The production of several other toxins is also indirectly controlled by this regulatory network, suggesting that other systems are also involved in toxin regulation. We have recently identified two new signal transduction systems that are involved in the regulation of several other toxins. Mutation of either system results in the attenuation of virulence when strains are tested in the mouse myonecrosis model. The aim of this project is to use of molecular microbiology and biochemical techniques to characterise these systems and subsequently elucidate their role in the regulation of toxin production.

#### **Role of regulatory genes in *Dichelobacter nodosus* (Dr Ruth Kennan and Prof Julian Rood)**

*Dichelobacter nodosus* is the causative agent of footrot, a debilitating disease of the feet of sheep. Known virulence factors of *D. nodosus* include the type IV fimbriae, which enable the bacteria to colonise the hoof and penetrate the lesion, and the production of extracellular proteases, which are capable of degrading the tissues found in the skin and hoof. However, little is known about how these and other potential virulence factors may be regulated. The *chp* gene cluster of *Pseudomonas aeruginosa* encodes a complex chemosensory system that controls twitching motility in response to environmental signals. A similar gene cluster has been identified in *D. nodosus*, but its role in the regulation of twitching motility or other virulence factors is unknown. This gene cluster contains six genes, *pilG*, *pilH*, *pilI*, *pilJ*, *chpA* and *chpC*. The aim of this project is to construct chromosomal mutants in several *pil* genes in the cluster and then to examine the effect of these mutations on twitching motility, fimbrial biogenesis and protease secretion.

### **Mechanism of conjugation in *Clostridium perfringens*** **(Dr Trudi Bannam & Prof Julian Rood)**

*Clostridium perfringens* causes gas gangrene, food poisoning and non-food borne diarrhoea in humans as well as various life threatening diseases in domestic animals. Many of the virulence factors implicated in these diseases are located on plasmids. Currently, there is a lot of interest in furthering our understanding of *C. perfringens* plasmid biology. We have identified several proteins that are required for conjugative transfer and, in particular have carried out extensive mutational analysis of TcpA, which is a putative coupling protein. These studies have shown that the last 60 amino acids of TcpA are important for its function. This project will aim to identify the amino acids that are involved in this process and to elucidate their functional role.

### **Mechanism of plasmid replication in *Clostridium perfringens*** **(Dr Trudi Bannam & Prof Julian Rood)**

*C. perfringens* causes gas gangrene, food poisoning and non-food borne diarrhoea in humans as well as various life threatening diseases in domestic animals. Many of the virulence factors implicated in these diseases are located on plasmids. Currently, there is a lot of interest in furthering our understanding of *C. perfringens* plasmid biology. We are studying a replication and maintenance region known to be encoded on a major virulence plasmid. Initial studies have identified the gene required for replication and a potential origin of replication. This project will involve cloning and mutagenesis of the *rep* region, overexpression of the Rep protein and DNA interaction studies to investigate the binding of the Rep protein to the origin of replication, as well as comparative analysis of other *C. perfringens* strains to determine the distribution of this plasmid replication system.

### **Functional biology of the *netH* and *netI* genes from a necrotic enteritis strain of *C. perfringens*** **(Dr Trudi Bannam, Dr Rob Moore (CSIRO), Dr John Boyce and Prof Julian Rood)**

Necrotic enteritis is an important disease of commercial chickens and causes significant economic losses in the poultry industry. The disease is caused by type A strains of *C. perfringens* and although the pathogenic mechanisms are not well defined recent collaborative studies in our laboratories have shown that  $\alpha$ -toxin is not an essential virulence factor. Three complete *C. perfringens* genome sequences are available and we have recently obtained an incomplete sequence of the genome of a virulent *C. perfringens* strain isolated from a case of necrotic enteritis. We have identified a genomic locus that is only found in chicken isolates of *C. perfringens* and carries three potential virulence genes. The aim of this project is to use genetic analysis to construct *netH* and *netI* mutants and to determine the phenotype effect of the resultant mutations.

### **Functional and structural analysis of the AprV2 and AprV5 proteases of *Dichelobacter nodosus*** **(Dr Xiaoyan Han, Dr Corrine Porter (Biochem & Mol Biol), Prof James Whisstock (Biochem & Mol Biol) & Prof Julian Rood)**

*Dichelobacter nodosus* is the principle causative agent of footrot, a highly contagious and economically significant bacterial disease affecting sheep in most countries. The extracellular proteases of this bacterium, AprV2, AprV5 and BprV, play an important role in the disease process. They are also involved in processing the preproteases into the mature enzyme forms. These enzymes contain a unique surface-exposed loop that

facilitates the formation of a substrate-enzyme complex and controls access of the substrate to the active site. This project will involve the mutagenesis of residues within the loop, with the objectives of determining their role in protease processing and enzyme specificity.

### **Functional biology of the Tn4451/3 family of mobile antibiotic resistance elements (Dr Vicki Adams, Dr Dena Lyras & Prof Julian Rood)**

The emergence of antibiotic resistant strains of bacterial pathogens is of major concern to public health authorities. It is important to understand how these determinants can spread from one bacterium to another, often across species barriers. In this laboratory we have been studying Tn4451 and Tn4453 from the pathogens *Clostridium perfringens* and *Clostridium difficile*. The transposition mechanism utilised by these elements involves the TnpX-dependent excision of the element as a circular intermediate that is not capable of independent replication and must be inserted back into a replicating DNA molecule to survive. Little is known about the specificity of TnpX, and by default Tn4451/3, for the target sites in either *C. difficile* or *C. perfringens*. The objective of this project will be to isolate numerous insertions in both clostridial species and to determine the sequences and specificity of the target sites in both species.

### **Genetics of toxin plasmids of *Clostridium perfringens* (Prof Julian Rood)**

*C. perfringens* is a potential bioterrorism agent because of its ability to produce potent extracellular toxins such as epsilon-toxin. Many of these toxins are encoded by genes that are located on large plasmids. As part of a larger project being carried out in collaboration with colleagues at the University of Pittsburgh and the University of California-Davis we are looking at genetic variation between these plasmids and are determining whether they are conjugative. This project will involve a combination of genetics and comparative genomics to determine whether toxin plasmids from type B, C and E strains of *C. perfringens* are conjugative.

### **Unravelling the pathogenicity of a “superbug” – how does *C. difficile* regulate the production of binary toxin? (Dr Glen Carter, Dr Dena Lyras & Prof Julian Rood)**

*Clostridium difficile* is a bacterial pathogen that is rapidly becoming the scourge of health services worldwide. It causes an array of intestinal diseases, ranging from mild self-limiting diarrhoea, to potentially fatal pseudomembranous colitis. Recent figures released from the United Kingdom indicate that whilst *C. difficile* and MRSA are responsible for similar numbers of deaths, the former causes significantly more morbidity and cost, with approximately 45,000 cases diagnosed in 2004. Whilst the two major virulence factors, Toxin A and Toxin B, have been studied in some detail, there has been scant research to date on how the other putative virulence factors, including a third toxin (CDT) are regulated. However, we have recently identified an orphan response regulator CdtR that regulates the expression of the binary toxin genes. This project aims to elucidate the mechanism by which CdtR carries out this process and will focus on the identification of the cognate sensor histidine kinase and structure-function studies on CdtR.

## Prof Ben Adler

Several vacancies

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[www.microbialgenomics.net](http://www.microbialgenomics.net)

### **Pathogenesis and molecular biology of leptospirosis.** **(Dr Gerald Murray, Dr Miranda Lo and Prof Ben Adler)**

*Leptospira* spp. are responsible for the most wide spread zoonosis in the world, as well as being a cause of disease in production and companion animals. Our determination of the genome sequence of one of the species has facilitated a genetic approach to understanding mechanisms of pathogenesis. Despite the disease prevalence and a worldwide distribution, the molecular mechanisms of pathogenesis in leptospirosis are poorly understood. This is largely due to the lack of genetic tools that can be used in *Leptospira*. This project will involve the generation and characterisation of *Leptospira* mutants using a recently developed transposon mutagenesis method. The specific focus of this project will be tailored to the interests of the student and which mutant(s) is chosen for analysis. Current areas of interest include: the role of chemotaxis in pathogenesis; development of vaccines against leptospirosis; microarrays to understand the regulation of virulence genes; the role of proteases in pathogenesis; the role of leptospiral LPS in pathogenesis; functional studies of leptospiral outer membrane proteins. The results of these studies will contribute to the understanding of *Leptospira* pathogenesis, and help to attribute function to the genome sequence, for which approximately half the open reading frames have no assigned function.

### **Pathogenesis in bacillary dysentery**

#### **Engineering toxinogenic chimeric derivatives of SigA - a cytopathic enterotoxin of *Shigella flexneri*** **(Prof Ben Adler)**

Enteric infections are a major cause of morbidity and mortality worldwide. *Shigella* infections alone result in over a million deaths annually, mainly in young children. We have shown in our laboratory that the enterotoxin SigA which resides on a pathogenicity island is a secreted cytopathic protease that contributes to intestinal fluid accumulation associated with *S. flexneri* infections. We have also shown that SigA binds to epithelial cells, degrades recombinant human alpha-fodrin *in vitro*, and cleaves intracellular fodrin *in situ*, suggesting that the cytotoxic and enterotoxic effects mediated by SigA are likely associated with the degradation of epithelial fodrin. This project aims to construct chimeric proteins where known or putative functional domains will be reciprocally exchanged between related toxinogenic molecules of the same family as SigA, namely Pat and EspC from pathogenic *E. coli*. Biologically active hybrids of SigA-Pet and SigA-EspC molecules will provide information which allows us to delineate domains that characterise these differences, in particular in determining substrate specificity and the cell binding domain. This will be achieved by switching several regions of SigA with those of either Pet or EspC to systematically identify discrete regions required for targeting. The hybrid toxins will be purified and evaluated initially for enzymatic activity using enzymatic assays, and the enterotoxicity of each toxin will be assessed in a rabbit ileal loop assay, a fodrin redistribution assay, and compared with the native SigA, Pet and EspC toxins. This

approach will identify domains involved in protein substrate specificity among this family of autotransporters in enteric pathogens.

## **A/Prof Brian M. Cooke**

Up to two vacancies  
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### **Haemoprotzoan parasite infections**

Research in our laboratory focuses on understanding the ways in which parasites of red blood cells cause disease and death in humans or animals. We aim to provide a friendly and helpful environment in which to gain knowledge and expertise in the process of modern biomedical research. Honours students will have the opportunity to design an original research project in one of our two major areas of interest in close consultation with their supervisors. Initially, students will be closely supervised and work side-by-side with a postdoctoral researcher. Importantly, you will acquire a wide range of skills including bioinformatic analysis, molecular techniques (cloning, PCR, Southern blotting, etc.), immunoblotting, immunofluorescence, tissue culture, biophysical assays, sub-cellular fractionation and proteomic analysis. Graduates will be well prepared to either enter the work force or begin a higher research degree.

### **Studies on malaria**

Malaria causes severe morbidity, mortality and socio-economic hardship particularly in Africa, South America and Asia. The disease is caused by protozoan parasites of the genus *Plasmodium*, with at least five species known to infect humans. Symptoms, including fever, chills, headaches and anaemia, are attributable to replication of parasites within red blood cells (RBCs) and vary in severity depending on the parasite species and the immune status of the host. In the case of falciparum malaria, serious complications can arise due to sequestration of parasitised RBCs (pRBCs) in the microvasculature of the brain or the placenta resulting in cerebral malaria and pregnancy associated malaria respectively. Research in our laboratory focuses on understanding the cellular and molecular mechanisms that underlie this phenomenon. The sequencing of the complete malaria genome has enabled us to identify a number of novel parasite proteins exported into the host RBC which we predict will be involved in the process of RBC modification. Using a range of techniques we are mapping interactions between these proteins and components of the RBC membrane skeleton. We are confirming the role that these interactions play in altered mechanical and adhesive properties of pRBCs by generating targeted gene knockout parasites. Our research is helping to better understand precisely how *P. falciparum* causes severe disease and will ultimately aid in the development of new drugs and vaccines.

### **Studies on babesia**

*Babesia bovis* is an important haemoprotzoan parasite of cattle that shows striking similarities with human malaria parasites. The disease is of major national and international importance and imposes huge economic burdens on the beef and dairy industries. A better understanding the basic biology of these parasites and the relationship between parasites and their host is required for the development of anti-parasitic vaccines,

drugs and new therapeutic regimens for this important disease. We are also interested in learning more about the basic biology of this parasite since it offers a unique opportunity to answer important questions about malaria infection that are not currently possible to perform in humans.

## **Prof Ross Coppel**

One to two vacancies

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### **Studies on malaria**

Malaria, caused by *Plasmodium falciparum*, is one of the most important infectious diseases of humans. Hundreds of millions of people are infected every year resulting in millions of deaths. This situation is likely to worsen significantly in coming years. I am interested in developing new methods of controlling infection, particularly through the design of new vaccines. Projects in my laboratory use a variety of approaches, both molecular and cellular to study the basic biology of the parasite and the relationship between parasite and host. Students working on each individual project will have an opportunity to learn and perform a wide variety of techniques during the course of their project.

### **Identification of important new malaria proteins using data from the malaria genome project.**

The entire genome sequence of *P. falciparum* is now available. We will use a number of bioinformatic analyses to select genes that may be involved in the invasion process or in the remodelling of infected red blood cells. Once candidate genes have been identified, the project will involve expression of these genes as proteins, using these to raise antibodies for use in a number of analyses to determine properties of these proteins and likely function in the parasite. We will also attempt to determine what other proteins, both host and parasite, are involved in interacting with the novel gene product.

### **Studies on the moving junction of malaria parasites**

*Joint project with Dr Karena Waller*

The Apicomplexan parasites *Toxoplasma* and *Plasmodium* share common invasion mechanisms, although they invade different cell types. Red blood cell invasion by *P. falciparum* parasites results from a series of co-ordinated events including parasite attachment and release of the invasion-related organelle contents. The moving junction (MJ) is formed at the juncture between the attached parasite and the red blood cell and moves along the sides of the invading parasite during invasion. Several proteins have been identified in the *T. gondii* MJ, but little is known about the composition of the *P. falciparum* MJ. This project will investigate the composition of the *P. falciparum* MJ and its functional role in red blood cell invasion. A variety of experimental techniques will be employed to localize MJ proteins in parasites and define their interactions with other proteins. Antibodies may also be assessed for ability to inhibit parasite invasion *in vitro*. Characterisation of *P. falciparum* MJ proteins will yield a more detailed model of parasite invasion and potentially new vaccine candidates.

## **Studies on protein-protein interactions in malaria**

It is known that particular malarial proteins are anchored to the membrane cytoskeleton of the infected red blood cell and to the surface of the parasite by protein-protein interactions with other membrane proteins. The interactions are important for invasion of red blood cells and for reproduction and survival of the parasite inside the red blood cell. We would like to study these interactions at the molecular level. To do this, the project will involve the production of the malaria protein by recombinant DNA technology. The produced protein will then be used in various binding assays to determine which human red blood cell proteins it binds to. By making successively smaller binding regions, the exact binding site will be mapped. The malaria protein will be added to permeabilised red cells and the effect of its binding on the mechanical properties of the red cell determined. This will enable us to zero in on specific domains of proteins that may be involved in important biological processes. With this information it may be possible to design specific peptide drugs that block these important interactions.

## **Seroepidemiology of malaria**

People in endemic areas eventually become immune to malaria after many infections. How does this occur? What immune responses do they develop that makes them immune? This project sets out to study the acquisition of protective antibody responses in patient groups exposed for the first time to malaria. Recombinant malarial antigens purified from prokaryotic and eukaryotic expression systems are used as the substrate for ELISA assays that detect levels and type of antibody. We are looking for a correlation between antibodies against a particular antigen and the immune state. This gives an insight into how people may be protected from malaria infection and helps in the design of new vaccines.

## **Testing of a novel malaria protein for its effectiveness as a malaria vaccine**

The genomic studies described above have led to the identification of a protein on the surface of the invading merozoite, MSP10. We would like to determine whether this protein has any capacity to act as a vaccine to protect against malaria infection. The project involves the construction of recombinant MSP10 protein or as a DNA vaccine and immunization of mice. The mice will then be challenged with malaria and their ability to resist infection measured. Techniques involve cloning, protein expression and purification, animal handling and measurement of parasitemia and immune responses by ELISA and immunofluorescence. The outcome will be the potential identification of a new malaria vaccine component.

## **Comparison of different methods of delivering a malaria vaccine**

We have identified a number of different malaria proteins that seem to be able to confer immunity to malaria in model systems. These proteins should make useful components of a subunit vaccine. For them to function most effectively it is important that they induce very high levels of antibodies. We would like to compare the immunogenicity of a number of different vaccine formulations including recombinant proteins, transgenic plants, DNA immunization and viral vectors and see which method or combination of methods induces the highest levels of effective antibodies. This would help us work out the optimum design of a malaria vaccine.

## **Nanovaccines against malaria**

*Joint Project with Prof Magdalena Plebanski in the Department of Immunology, Monash University.*

Vaccines utilizing nanotechnology have become a very active and exciting area of adjuvant and vaccine carrier research. With increasing demand on prevention and treatment for an increasing number of diseases, developing vaccines against various diseases also become one of primary goals for healthcare. This project will make use of the nanopatform technology developed in Professor Plebanski's laboratory, using nanoparticles as vaccine carriers and adjuvant to induce unusually strong cellular and hormonal immune responses. A range of important target antigens have been identified in the malaria parasite and DNA constructs with multiple targets have been administered as DNA vaccines. The combination of such DNA vaccines, protein or whole parasite based vaccines and nanotechnology offers great potential for the development of a new generation of effective malaria vaccines. This project will use test the capacity of various malaria nanoparticle formulations to protect mice from infection by strains of murine malaria. The project will involve preparation of the vaccines, measurement of the induced cellular and antibody responses and performance of the mouse challenge experiments.

## **Studies on tuberculosis**

Tuberculosis is the leading cause of death in the world from a single infectious disease. Little is known about the mechanisms of pathogenesis of *Mycobacterium tuberculosis* and the current vaccine does not afford complete protection. After a century of decline, tuberculosis is increasing and drug-resistant strains have emerged. This bacterium is well adapted to survival within its human host and can resist the bactericidal actions of the immune system. *M. tuberculosis* is naturally resistant to many antibiotics and now, in addition, many strains have acquired resistance to drugs used specifically to treat TB patients. The bacterial cells are able to withstand immunological and chemical attack, partly because they have very robust cell walls. We are studying the genetics and biochemistry of the mycobacterial cell walls with the ambition of developing new drugs to combat TB.

## **Targeted mutation of mycobacterial genes**

Using bioinformatics analyses of the TB genome, we will attempt to identify genes likely to be involved in the biosynthesis of particular mycobacterial cell wall structures. These genes will be disrupted using allelic exchange methods and the resulting mutant bacteria characterized for changes in the cell wall.

## **Microarray analysis of mycobacteria**

The various mutants produced in the preceding projects may have altered patterns of gene expression as they try to cope with loss of important functions. The genes that have altered expression as a compensatory mechanism are likely to be involved in pathways related to those lost in the mutant. We have constructed a mycobacterial DNA microarray that will be used for the analysis of these mutants. RNA extracted from mutant strains will be compared to RNA from wild-type cells to identify genes of importance in cell wall biosynthesis processes.

Two vacancies

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### **The molecular mechanisms by which *Helicobacter pylori* causes stomach cancer**

*Helicobacter pylori* (*Hp*) is a prototype of cancer-inducing pathogen. This motile rod-shaped Gram negative bacterium colonises persistently in the human stomach, causing chronic gastritis and gastric cancer in susceptible individuals.

Virulent *Hp* expresses a Type IV secretion system (T4SS), a major virulence factor which functions as macromolecular machine gun that “shoots” virulence proteins and peptidoglycan molecules into the host cells. Recently, we discovered that a novel adhesin of *Hp*, CagL, is expressed on the surface of T4SS and is able to dock onto integrin receptors on human gastric epithelial cells, turn on integrins and simultaneously trigger the secretion of other virulence molecules into the stomach cells. Once intracellular, the *Hp* virulence factors including CagA and peptidoglycan then interact with specific host signalling molecules to trigger activation of host tyrosine kinases, nuclear factor kappa B (NF $\kappa$ B) and/or downstream proinflammatory responses such as the secretion of cytokines. Meanwhile, the vacuolating toxin secreted by *Hp* dysregulates normal host cell functions, causes severe cytotoxicity and disrupts the gastric epithelium. The molecular basis of how *Helicobacter* infection progresses into cancers however remains largely a mystery.

Our lab is interested in using a multi-disciplinary approach to understand the pathogenesis of *Helicobacter*-associated malignancies. Projects are available to address the following exciting questions:

- How does the *Hp* protein CagL function as a molecular switch to turn on Type IV secretion?
- Can we utilise the Type IV secretion of *Hp* for delivery of therapeutic proteins?
- How does CagL modulate integrin signalling in the gastric cells to cause diseases?
- Which other host proteins does *Hp* interact with during the different stages of infection?
- What are the virulence factors of *Hp* which trigger inflammation and carcinogenesis?
- How does *Helicobacter* turn normal host cell signalling pathways into oncogenic cascades?

The honours project will enable hands-on experience with mutagenesis, bacterial culture, eukaryotic cell culture techniques, RNAi, immunostaining, Western blotting, ELISA, confocal laser scanning microscopy, live cell imaging, etc. Someone who is enthusiastic in learning about the exciting secrets of bacterial pathogenesis, bacteria-host interactions and infectious cancer biology is welcome to apply.

Two vacancies

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**Virulence and hypervirulence genes of *Clostridium difficile*  
(Dr Dena Lyras, Dr Glen Carter & Prof Julian Rood).**

*Clostridium difficile* is recognised as the major cause of nosocomial diarrhoea in Australian hospitals. Chronic colitis syndromes caused by this organism are a significant cause of morbidity in the hospital system and control and treatment costs approximately \$1 million per hospital per year. The recent emergence of hypervirulent strains has increased the severity of disease and hence the urgency with which the mechanism of disease needs to be understood. The pathogenesis of *C. difficile*-associated diseases involves the production of two large cytotoxins, Toxin A and Toxin B, as well as a number of other toxins and virulence factors. However, there is considerable variation between disease-causing strains with regard to factors associated with virulence. This project will involve the analysis of virulence factors and vegetative cell or spore surface exposed antigens of virulent and hypervirulent strains of *C. difficile*.

**The pathogenesis of infections caused by *Clostridium sordellii* - how does *C. sordellii* cause disease in post-abortive, post-partum and transplant patients?  
(Dr Dena Lyras, Dr Milena Awad, Dr Glen Carter & Prof Julian Rood)**

The toxigenic anaerobic bacterium *Clostridium sordellii* is an emerging human pathogen that causes rapidly progressing tissue necrosis, shock, a characteristic immune response and multi-organ failure. It has a very high mortality rate of approximately 70%, reaching 100% for postpartum patients, and has been associated with infections following spontaneous or medically induced abortion, notably following the administration of mifepristone (RU486). Little is known about how *C. sordellii* causes disease. We have now developed methods for carrying out genetics in *C. sordellii* making it possible to use molecular approaches to explore the role of putative virulence factors in disease. The approach will be to construct mutants of *C. sordellii* strains and characterise the isogenic wild-type and mutants using appropriate assays. In particular, we will determine the role played by TcsH in the disease-causing ability of a *C. sordellii* strain by constructing chromosomal mutations in this gene and analysing the resultant strains. The successful completion of these experiments will enable the precise role and importance of the toxins encoded by *C. sordellii* in disease to be determined.

**Genetic and phenotypic characterisation of virulence factors encoded by human isolates of the bacterial pathogen *Fusobacterium necrophorum*.  
(Dr Dena Lyras & Prof Julian Rood)**

*Fusobacterium necrophorum* is a Gram negative anaerobic bacillus that can be the primary pathogen causing either localised abscesses and throat infections such as tonsillitis or systemic life-threatening disease such as Lemierre's disease. The role that this bacterium plays in the former has only recently become evident. This bacterium is also an important primary and secondary pathogen in farm animals. The leukotoxin of *F. necrophorum*, encoded by the *lktA* gene, is considered to be of pre-eminent importance in the pathogenesis of invasive infections in animals and humans. However, recent studies

have shown that many human isolates do not encode this toxin. The role that leukotoxin plays in infections has not been directly determined because genetic manipulation of this bacterium has not been reported. In this research project, we will apply our current methods for the genetic manipulation of anaerobic bacteria to *F. necrophorum* and attempt to mutate the *IktA* gene. We will also determine the genome sequence of a human isolate and identify potential virulence factors. We will also collect Australian isolates from diverse sources and determine the virulence profile of each strain to gain a better understanding of local strain diversity of *F. necrophorum*.

## A/Prof Anna Roujeinikova

Two vacancies

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### **Investigation of structure and dynamics of *Helicobacter pylori* motility protein B.**

The aim of this project is to understand the relationship between the structure, dynamics and function of a key component of the bacterial flagellar motor, the motility protein B (MotB). Motility is essential for the survival, chemotaxis and virulence of many pathogenic bacteria, including the chosen model system, the carcinogenic bacterium *Helicobacter pylori*. Bacterial motility, and the MotB function in particular, can be used as an unconventional antibacterial target to cure or prevent disease. From the point of view of nanotechnology, knowledge about how the bacterial flagellar motor works may enable us to discover nature's blueprint of a nanoscale engine and learn how bacterial cells convert electrochemical energy into mechanical energy of rotation. Progress in this area has so far been hindered by the lack of detailed structural information about motility proteins, and we aim to address this gap in knowledge. We have recently determined the first crystal structure of the MotB domain that anchors the proton-motive-force generating mechanism of the bacterial flagellar motor to the cell wall, and formulated a model of how the stator attaches to peptidoglycan. We now aim to solve X-ray crystallographic structures of the full-length MotB and a series of N-terminally truncated MotB variants and to characterise the dynamics of this protein using a peptide amide hydrogen/deuterium exchange coupled with liquid chromatography and mass spectrometry.

### **Structure-based design of antibacterial peptides targeting molecular chaperone DnaK from *Helicobacter pylori*.**

The carcinogenic bacterium *Helicobacter pylori* infects billions, and about 10% of the *H. pylori*-infected population develops severe gastroduodenal diseases in adulthood. The infection can be treated with a combination of a proton pump inhibitor with two broad-spectrum antibiotics, but *H. pylori* readily develops resistance to the currently used antibiotic components. This study aims to design selective, peptide-based, antibacterial inhibitors of the molecular chaperone DnaK from *H. pylori* as a new strategy for *H. pylori* eradication. These inhibitors would be able to kill the bacterium by stalling its essential protein repair machinery. In collaboration with M. Liebscher (Germany), we have recently solved the first crystal structure of an *E. coli* DnaK fragment with a peptide-derived inhibitor and elucidated the molecular mechanism of inhibition at the structural level. We now aim to exploit the differences between the substrate binding sites of *E. coli* and *H. pylori* DnaKs in structure-assisted design of *H. pylori*-specific inhibitor peptides. The work will involve

gene cloning, protein expression, purification and crystallization, X-ray crystallography and computer modelling.

## Dr Anton Peleg

Several vacancies

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### **Use of *Caenorhabditis elegans* to study prokaryote-eukaryote interactions**

The soil dwelling nematode, *C. elegans*, has been used as a non-mammalian model system to study host-pathogen interactions over the last 10 years. Given its small size, relative ease of handling, and low cost, it is well suited for large scale screening of microbial mutant strains. Most recently, we extended the use of the *C. elegans* model to study a polymicrobial infection between the human fungal pathogen, *Candida albicans*, and the Gram-negative bacterial pathogen, *Acinetobacter baumannii*. We identified that *A. baumannii* inhibits several key virulence determinants of *C. albicans*, specifically filament and biofilm formation. The focus of this project is to understand the molecular mechanisms by which *A. baumannii* antagonizes *C. albicans*. This will be achieved through the use of an *A. baumannii* random mutant library created using a Tn5 transposon. Rescue cloning will be used to identify the disrupted genes of *A. baumannii* mutants that are unable to inhibit *C. albicans* filamentation after co-infection in the worm. Targeted mutagenesis techniques and gene complementation will then be used to confirm the significance of the identified genes. Understanding the defense mechanisms used by competing microbes is an innovative approach in identifying biological and virulence pathways with therapeutic potential. Furthermore, *A. baumannii* virulence determinants toward *C. albicans* (unicellular eukaryote) may provide important insights into *A. baumannii* virulence toward mammals.

### **Characterizing the pathogenic significance and gene regulatory function of the *gacS*-like sensor kinase gene and its putative response regulator (*gacA*) in *Acinetobacter baumannii*.**

*A. baumannii* has emerged worldwide as a highly troublesome, hospital-acquired Gram-negative organism. Despite causing a wide range of serious hospital-acquired infections, the pathogenic mechanisms of the organism are poorly understood. Through a pilot screen, we identified that the *A. baumannii gacS*-like sensor kinase gene was important for its virulence toward the unicellular eukaryote, *C. albicans*. This gene codes for the sensor (histidine kinase) of a highly conserved two-component regulatory system found in many Gram-negative pathogens. Importantly, homologs of the *A. baumannii gacS* gene have been shown to regulate key virulence parameters in other organisms, including quorum-sensing and biofilm formation in *P. aeruginosa*, bacterial invasion in *Salmonella*, and toxin production in *Vibrio cholerae*. Furthermore, inactivation of these genes leads to attenuation in virulence toward a range of hosts, including fungi, plants, worms, and mammals. Thus far, the pathogenic significance of the *A. baumannii gacS*-like sensor kinase gene is unknown. The aim of this project is to construct targeted knockout mutants and complemented strains to assess the significance of the *A. baumannii gacS/gacA* system for biofilm formation, quorum-sensing gene expression (using real-time PCR), and virulence in a mammalian system. Subsequent work will characterize the genetic regulatory function of the *gacS/gacA* genes as determined by microarray analyses.

## **Interface between antibiotic resistance and virulence in *Staphylococcus aureus***

*S. aureus* is one of the most common human bacterial pathogens, and is able to cause a wide range of life-threatening infections in the community and hospital setting. As a consequence of the rising rates of methicillin-resistant *S. aureus* (MRSA), agents such as vancomycin and daptomycin have been increasingly relied upon. Unfortunately, reduced susceptibility to these agents has now emerged. Interestingly, resistance to these agents leads to marked phenotypic changes to the bacterial cell, characterized by thickening of the cell wall and changes in lipid composition and electrical charge. We have identified that such changes may be associated with reduced virulence in a non-mammalian infection model (*Galleria mellonella*). To characterize the genetic evolution of resistance to vancomycin and daptomycin, we have performed whole genome sequencing of pairs of clinical *S. aureus* isolates whereby the first isolate is susceptible and the paired isolate is non-susceptible to each agent. Intermediates have also been sequenced. Through use of comparative genomics, single point mutations have been identified. The aim of this project is to confirm the mutations identified from comparative genomics and construct targeted knockout mutants of genes with a putative virulence function. The constructed strains will then be tested in a mammalian model of infection (murine tail vein injection).

### **Dr John Boyce**

Two vacancies

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## **Melioidosis: intracellular survival and virulence of *Burkholderia pseudomallei***

*Burkholderia pseudomallei* is the causative agent of melioidosis, a potentially fatal disease of humans that is endemic in South East Asia and tropical parts of Australia. Little is known about the molecular mechanisms of *B. pseudomallei* virulence and we are interested in identifying novel bacterial virulence factors that may be candidate vaccine antigens or targets for antimicrobial drugs.

## **Investigation of the molecular mechanisms associated with actin-based motility in *B. pseudomallei* (Dr Elizabeth Allwood, Prof Ben Adler and Dr John Boyce)**

*Burkholderia pseudomallei*, has the ability to invade and proliferate within phagocytic and non-phagocytic host cells. After internalization, *B. pseudomallei* escapes from endocytic vacuoles into the cytoplasm where it induces actin polymerization, allowing it to move within and between cells. Little is known about the molecular mechanisms of *B. pseudomallei* actin-based motility; this study aims to elucidate the bacterial and eukaryotic factors involved in actin polymerization and actin-mediated motility. The *B. pseudomallei* BimA protein is absolutely required for actin polymerization. This project will initially use immunoprecipitation assays to uncover critical protein-protein interactions between BimA and other *B. pseudomallei* and eukaryotic proteins. We will utilise cell invasion assays and confocal laser scanning microscopy to screen a *B. pseudomallei* transposon mutant library for defects in actin-based motility. This will allow the identification of bacterial genes critical for actin-based motility. These studies will provide a

better understanding of the molecular mechanisms associated with actin-based motility in this pathogen which will allow the identification of potential live attenuated vaccine strains and/or potential targets for rational drug design.

### **Pasteurella multocida: defining the mechanisms of pathogenesis and immunity**

*Pasteurella multocida* is a Gram-negative bacterial pathogen that causes a number of different diseases in cattle, pigs and poultry resulting in serious economic losses worldwide in food production industries. We are interested in understanding the molecular mechanisms of pathogenesis in this bacterium with an aim to developing new, more effective and widely applicable vaccines or antimicrobial drugs. Our focus is on understanding the virulence characteristics of the surface of the bacterium as this is the primary site of interaction between the bacteria and the host.

#### **Defining critical strain-specific surface expressed virulence factors (Dr Marina Harper, Prof Ben Adler and Dr John Boyce)**

We have access to a range of *Pasteurella multocida* strains with different levels of pathogenicity or which cause disease in different animal hosts. New genomic technologies have allowed the rapid sequencing of bacterial genomes and we have used high-throughput short read sequencing to determine 99% coverage genome sequences of a range of *P. multocida* strains. This project will initially use bioinformatics analyses to identify genes encoding strain-specific surface expressed proteins. We will then use mutagenesis techniques to inactivate selected surface proteins and test the effect of these mutations on strain virulence. These experiments will help identify strain-specific virulence determinants and help to elucidate the role of surface proteins in *P. multocida* host specificity.

#### **Global regulation of virulence in *P. multocida* (Dr Xenia Gatsos, Dr John Boyce and Prof Ben Adler)**

Virulent *P. multocida* strains produce a polysaccharide capsule which is a critical virulence factor and important determinant of host specificity. We have recently shown that capsule expression is regulated at the transcriptional level by a nucleoid-associated protein. Furthermore, we have used DNA microarrays to show that a range of other virulence factors are co-regulated with capsule. In this project we will use gel-shift assays, together with directed and random mutagenesis, to determine the molecular mechanism by which the regulatory protein binds DNA and regulates promoter activity. We will also test the importance of the genes co-regulated with capsule in virulence in *P. multocida*. These studies will identify novel *P. multocida* virulence factors and allow the design of new antimicrobial and vaccine strategies.

### **Antibiotic resistance and virulence in *Acinetobacter baumannii***

*A. baumannii* is an important nosocomial human pathogen worldwide. Over the last decade this bacterium has shown an unparalleled increase in antibiotic resistance with numerous studies reporting the occurrence of multidrug resistance (MDR). Disturbingly this includes reports of resistance to frontline antibiotic therapies. As a consequence *A. baumannii* is recognised as one of the six top-priority MDR pathogens worldwide.

**Understanding antibiotic resistance in *A. baumannii*.  
(Dr John Boyce and Dr Marina Harper)**

Membrane bound efflux systems play important roles in the survival of Gram-negative bacteria. Their main function is the removal of metabolic products and toxins, such as antibiotics, from the cell. Clinically important efflux systems include the resistance-nodulation-cell division (RND) superfamily and the multidrug and toxin extrusion (MATE) family of transporters. RND and MATE-family proteins have been reported in *Acinetobacter*. However, their role in antibiotic resistance is little studied and remains undefined. This project will use bioinformatics analyses of *A. baumannii* genome sequences to identify putative efflux proteins. These analyses will be complemented with transcriptional studies to identify genes specifically regulated in response to antibiotics. The function of the putative efflux proteins will be confirmed by directed mutagenesis of *A. baumannii*. These results will significantly increase the knowledge of the antibiotic resistance mechanisms of *A. baumannii* and form the basis of future antimicrobial drug development projects.

**Identifying virulence genes in *A. baumannii*.  
(Dr John Boyce, Dr Marina Harper, and Dr Jian Li)**

Despite its critical medical importance, very little is known about virulence factors in this pathogen. This project will use a range of widely applicable biomedical research techniques, including bioinformatics analyses of the *A. baumannii* genome and directed and random transposon mutagenesis, to identify and characterise genes involved in pathogenesis and antibiotic resistance. We will initially focus on predicted surface associated virulence factors including lipopolysaccharide and capsule. These studies will define novel virulence factors in this pathogen and these results will form the basis of future vaccine and/or antimicrobial drug development projects.

## PROJECTS BASED AT AFFILIATED INSTITUTIONS

### CSIRO Livestock Industries

The Australian Animal Health Laboratory (AAHL) is a national centre of excellence in disease diagnosis, research and policy advice in health. AAHL is one of the most sophisticated laboratories in the world for the safe handling and containment of diseases and plays a vital role in maintaining Australia's capability to quickly diagnose exotic and emerging diseases. AAHL includes a high-biocontainment facility, to safely fulfill its major role of diagnosing emergency disease outbreaks and its role in conducting cutting-edge research essential to the success of health related research in Australia.

All projects in our research adopt a multi-pronged approach to finding solutions to problems, with this in mind, the project areas provide candidates with experience in a broad range of techniques from molecular biology techniques to cell culture and immunoassays.

For more information visit: <http://www.csiro.au/places/aahl.html> or contact CSIRO, AAHL, Phone 5227 5000

N.B. These projects would be undertaken at the Australian Animal Health Laboratory, CSIRO Livestock Industries, 5 Portarlington Rd., East Geelong, 3220.

### Dr Wojtek Michalski, Dr James Wynne and Dr Dieter Bulach

1 vacancy

([Wojtek.michalski@csiro.au](mailto:Wojtek.michalski@csiro.au))

#### **A comparative genome analysis of human and animal *Mycobacterium avium* subspecies *paratuberculosis***

Crohn's disease is a significant health issue in Australia. It is estimated that over 28,000 Australians have Crohn's disease. Despite considerable research the cause of Crohn's disease remains unclear. One of the most widely, and often contentiously debated hypothesis suggests that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) – a gram positive acid fast bacilli known to cause a similar condition in ruminates and often found in dairy products – is the primary cause of Crohn's disease. Recently (in a world first), we have successfully isolated and cultured MAP from juvenile Crohn's disease patients. Subsequent to this success we have embarked on an ambitious project to sequence the entire genome of all MAP isolates obtained from this patient and compare these with the genomes of MAP isolates derived from animal sources. All genomes have been sequenced using next-generation DNA sequencing technology. In this honours project the student will work to validate and further characterise a number of biological important genetic differences between the *Mycobacterium* isolates identified through the whole genome sequencing effort. The student will use a systems biology approach by initially focussing on genetic variation and then examine the consequential protein and biological variations. Within this project the student will first employ a range of molecular biological techniques to compare genetic variation between *Mycobacterium* isolates. These techniques include, DNA and RNA isolation, PCR, cloning, DNA sequencing and

bioinformatics. To examine the consequential protein changes the student will use SDS-PAGE electrophoresis, western blotting and mass spectrometry.

**Dr Linfa Wang, Dr Dieter Bulach and Dr Michelle Baker**

1 vacancy

([dieter.bulach@csiro.au](mailto:dieter.bulach@csiro.au))

### **Antiviral immune mechanisms in bats**

Bats have been identified as the natural host reservoir for a variety of viruses, several of which have had a significant impact on human and animal health, tourism, and trade. Although bats may be persistently infected with many viruses, they rarely display clinical symptoms of disease. Despite the central role of bats in harbouring and transmitting viral diseases, there is currently little information available on the immune system of bats and no reagents exist to study their immune response. This project will focus on the identification and characterization of genes involved in the innate immune response of the black flying fox and on the development of reagents to examine the immune response of bats to viral infection. This project will involve the identification and characterization of genes believed to play a role in antiviral immunity in the black flying fox and will involve a wide range of bioinformatics, molecular (PCR, cloning, sequencing) and protein techniques (expression, antibody production).

## **Burnet Institute**

### **A/Prof Johnson Mak**

Up to three vacancies

Centre for Virology, The Burnet Institute, 85 Commercial Road, Melbourne, 3004, Phone 9282 2217

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The Mak lab aims to better define the replication processes of HIV-1 and its closely related viruses through basic research, and to identify critical features that can be exploited for translation to novel antiviral strategies for clinical applications. The successful candidate should be enthusiastic about HIV research, and a wide range of molecular virology techniques will be taught in these projects.

### ***Visualizing the movement of HIV-1 genetic materials and proteins in infected cells***

Viruses are known to hijack some of the seemingly unrelated host cell machineries to further their own propagations. Investigations of virus replication often enable us to learn a great deal about the cell biology of the host cells. Advancement of imaging technology in recent time has allowed us to visualize the movement of viral components and their interactions with host cell machinery in real-time, which has enriched our understanding on the interplay between virus, host and the underline mechanism of the pathogenicity of virus. We have established a state-of-the-art deconvolution microscope, which provides us the means to track of the movement of viral components in HIV-1 infected cells in real time. The objective of this project is to use fluorescent technology to unravel the process of

HIV-1 assembly, and to understand the interplay between virus and host during virus formation.

### **Differential requirement of uracil DNA glycosylase (UNG2) between X4- and R5- HIV-1 infection in primary cells**

The process of retroviral uncoating, which leads to reverse transcription and integration is poorly understood. Cellular protein, uracil DNA glycosylase (UNG2, a cellular DNA repair enzyme), has been implicated to be involved in this process. However, the precise role of UNG2 in HIV-1 biology remains controversy as data derived from different model systems have led to contradictory interpretations. Using an array of molecular virology techniques, we have found that UNG2 is not required for X4- but is needed for R5-tropic HIV-1 to infect primary T-lymphocytes and MDMs, which provides direct evidence to reconcile the current dispute over the role of UNG2 in HIV-1 replication. The focus of this project will be to dissect the differential mechanistic contribution of UNG2 between R5- and X4-tropic HIV-1. Successful completion of this proposal will uncover novel information toward our understanding in HIV-1 biology, and help to define the interplay between host cell and virus during HIV-1 infection.

### ***Structural biology of HIV-1 assembly***

In the process of virus formation, viral genomes and proteins interact with a series of host factors in an orderly fashion for the generation of infectious virus particles. During this process, viral genomes and proteins undergo a series of structural rearrangement to achieve a series of steps along the way. These folding and re-folding of RNA and protein structures expose a number of binding pockets that could potentially be targeted for the design of novel antiviral agents. The three-dimensional structures of these viral genomes and proteins can be revealed using structural biology techniques, such as X-ray crystallography and small angle x-ray scattering. Successful completion of this project will lay the foundation of rational drug design during HIV-1 assembly.

### ***Diversity of HIV-1***

One of the hallmarks of HIV-1 infection is the generation of diverse quasispecies that exhausts, and ultimately cripples, the immune system of the host. Conversely, limited viral diversity is associated with reduced viral pathogenicity. The rapid evolution of HIV-1 is arguably its strongest counter-measure to neutralize host immune pressure and anti-retroviral assault. Little effort has been placed to develop an inhibitor that directly constrains viral diversity. While the infidelity of HIV-1 reverse transcriptase introduces mutations into the viral genomes, it is retroviral recombination that drives viral evolution and furthers the diversity of HIV-1. It has been shown that the structure of viral RNA genome is important determinant of the retroviral recombination process, and recombination hotspots are likely to exist within the viral genome. The objective of this proposal is to use fluorescent HIV-1 to dissect the molecular mechanism of this process, and to identify leads for the development of novel antiviral therapeutics.

### ***HIV latency***

Viral latency is one of the key obstacles to prevent highly active antiretroviral therapy to attain complete suppression of HIV replication in patients, which has posted a major challenge in the clinic to eradicate the last trace of virus in the blood stream from patients. A latently infected cell will not actively produced virus, and will remain dormant to evade

immune and antiviral surveillance until the environmental conditions are desirable for virus production and propagations. Using a primary cell infection system to mimic the viral replication and dynamics *in vivo*, we have identified a sub-population of HIV infected cells that will undergo latency after productive infection. The objective of this study is to characterize this sub-population of infected cells to delineate the mechanism that regulate viral latency. Unveiling the mechanism of viral latency will provide the necessary tools that can be used to 'flush out' all the residual HIV infected cells to eradicate HIV infection in the patients.

**A/Prof Gilda Tachedjian, Dr Con Sonza and Dr Jenny Anderson**

Two vacancies

Molecular Interactions Group (MIG)

Macfarlane Burnet Institute for Medical Research and Public Health

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MIG web site: <http://www.burnet.edu.au/home/cvirology/molecularinteractions>

The Molecular Interactions Group studies interactions between viral proteins and host cell factors that promote or prevent replication of human immunodeficiency virus type I (HIV-1) which causes acquired immune deficiency syndrome (AIDS) and affects approximately 33.2 million individuals worldwide. The laboratory also has a strong focus on the study of drug resistance mutations in the HIV-1 reverse transcriptase (RT) and the development of microbicides to prevent the sexual transmission of HIV.

Note that all projects will require the student to work with HIV in a PC3 laboratory.

### **Silent Mutations in the HIV-1 Reverse Transcriptase Selected During Antiretroviral Therapy (C. Sonza and G. Tachedjian)**

New drug targets and strategies are needed for the inhibition of HIV-1 due to the eventual emergence of drug-resistant strains to current antiretroviral agents. A successful target for anti-HIV-1 drugs has been the HIV reverse transcriptase (RT). Analysis of an extensive and unique database containing over 20,000 HIV-1 genotype sequences has led to the identification of synonymous or silent mutations in the HIV-1 RT at codons 65 and 66, which are more prevalent in patients on antiretroviral therapy compared to drug naïve individuals. In this project we will determine the role of these mutations in the evolution of drug resistance and viral fitness. These studies are important in order to develop more effective antiretroviral agents and treatment strategies for HIV infected individuals.

### **Characterization of a Natural Microbicide against Human Immunodeficiency Virus (C. Sonza and G. Tachedjian)**

The joint United Nations Program on HIV/AIDS estimates that 33.2 million people were infected with HIV in 2007 and 15.4 million of these were women. In sub-Saharan Africa, women represent more than 50% of the people living with HIV/AIDS. HIV is transmitted by sexual intercourse. Condom use and male circumcision have been shown to be effective in preventing HIV infection. However, these are under the control of men and in some

cultures, negotiating their use is difficult for women. One strategy that has received considerable attention in the last decade is the development of topical microbicides that can be applied by women to prevent the sexual transmission of HIV and other sexually transmitted infections (STIs). There is evidence to suggest that lactic acid, produced in the healthy female genital tract by vaginal lactobacilli, may act as a natural microbicide. In this project we will determine whether lactic acid is able to kill (virucidal activity) different clades of HIV-1 and HIV-2. The mechanism by which lactic acid inactivates HIV will also be determined. This study could lead to strategies to decrease the acquisition of HIV by women.

### **Impact of cellular APOBEC3F on HIV-1 trafficking and replication (J. Anderson and G. Tachedjian)**

Host cells contain various intracellular defences to protect themselves from invading pathogens. These intracellular defences include the recently defined cellular APOBEC3 proteins that protect cells from reverse transcribing pathogens. For instance, APOBEC3F (A3F) and APOBEC3G (A3G) potently restrict the devastating human retrovirus, HIV-1. These proteins commonly hypermutate retroviral DNA during reverse transcription and perturb early events in retrovirus replication to block retroviruses in target cells. However, their precise restriction mechanisms remain unclear and controversial. This project will build on our work with A3G (Anderson et al 2008 *Virology* 375:1-12) and define how A3F perturbs the trafficking and early replication of HIV-1 in target cells. This will require virology, molecular biology, cell biology and fluorescent deconvolution microscopy techniques. Importantly, defining A3F restriction mechanisms may expose new strategies or targets in HIV-1 replication for developing new antiviral therapies, which are clearly needed given current problems with drug resistance and toxicity.

### **Host Cell Proteins Required for HIV Replication (C. Sonza and G. Tachedjian)**

Viruses, including HIV-1, augment their relatively limited genetic capacity by hijacking the host cell machinery for their replication. The extent of involvement of the host cell machinery is dramatically underscored by a recent genome-wide siRNA knockdown study demonstrating that more than 250 host cell factors are required for HIV replication (Brass et al *Science* 2008 319:921). Using a yeast two-hybrid (Y2H) screen we have found that the host cell protein, Golgi-associated ATPase enhancer of 16kDa (GATE-16) interacts with the HIV reverse transcriptase (RT). In addition, the abovementioned siRNA knockdown study showed that GATE-16 is required for HIV replication however, the precise role of GATE-16 in the virus life-cycle remains to be elucidated. In this project we will perform siRNA knockdown studies to determine the role of GATE-16 and related proteins in HIV replication. These studies are important to understand the basic replication strategy of HIV and to identify potential drug targets.

Two vacancies

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Millions of children are dying from malaria each year and more than one third of the world's population are at risk for infection with the most virulent malaria parasite, *Plasmodium falciparum*. A broadly effective malaria vaccine would be a major triumph for global public health yet years of research and clinical trials are yet to deliver such a vaccine. One of the major obstacles to this is the extraordinary genetic diversity and rapid evolution of the parasite. Our team is analysing field isolates from PNG to understand the population biology of the malaria parasite with a particular focus on the diversity of the surface antigens that vaccines are modelled on.

### **A framework for the development of next generation malaria vaccines**

Previous studies in our laboratory investigating the range and distribution of diversity of a number of leading malaria vaccine candidates in Africa, Asia and the Americas have shown that not only is there extensive diversity, there are significant differences among these regions suggesting that malaria vaccines may need to be customised in a manner similar to the Influenza vaccine. Similar molecular data is lacking for the Pacific region. To investigate vaccine candidate diversity in the Pacific, the project will involve collecting DNA sequences of vaccine antigens from the *P. falciparum* field samples from PNG. A pipeline of sophisticated bioinformatic and population genetic analyses will then define the range, distribution and dynamics of antigen diversity. This will provide a rational framework for the design of malaria vaccines in the region and a basis for monitoring their effects on the parasite population during vaccine trials.

### **Var gene diversity and naturally acquired immunity to malaria**

Humans naturally exposed to *P. falciparum* eventually develop a non-sterilising immunity against all of the clinical symptoms of the disease by early adolescence. Uncovering key steps in the development of this natural immunity may lead to malaria vaccines that could mimic this critical natural exposure. We are investigating patterns of antibody acquisition in children that are actively developing immunity to malaria using protein arrays. The project will involve the critical first step to generating protein arrays by assessing the diversity of the major surface antigen (*var*) genes of *P. falciparum*. *Var* gene repertoires will be sampled from field isolates using a framework previously developed in the lab (Barry *et al.* 2007 PLoS Pathogens 3(3):34). Combining the sequence data with that from previous studies in the lab will provide a novel insight into the fine scale spatial and temporal distribution of *var* gene diversity in PNG, mechanisms of evolution in the gene family and their potential as vaccine candidates.

## Dr. Shuo Li, A/Prof. Bruce Loveland and Prof. Eric Gowans

One Vacancy

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### Does hepatitis C virus infection affect dendritic cells?

Hepatitis C Virus (HCV) persistently infects ~3% of the world population, leading to cirrhosis, cancer and liver failure. It is not well understood why the human immune system often fails to clear the virus, although it is likely to be multi-factorial. We are studying the identity and function of HCV-specific natural regulatory T lymphocytes (Treg). The influence of these cells on the outcome of HCV infection is unclear, although in chronically infected patients they appear to be far more abundant than IFN $\gamma$ -producing effector T cells (Teff). One clue to why Treg cells accumulate may be in the function of Dendritic Cells in the presence of a chronic virus infection. Dendritic cells play an essential role in the induction and maintenance of effective immune responses, however their functions can be affected by pathogens.

We found that monocyte-derived DC (Mo-DC) from HCV patients were less responsive to a laboratory HCV strain than Mo-DC prepared from healthy donors. Specifically, whereas 170 genes were differentially expressed by Mo-DC before and after HCV exposure in healthy donors, only 9 genes were up- or down-regulated in Mo-DC prepared from patient samples. We are following up the clues from our microarray data, to discover the implications for DC function in HCV infection.

The proposed project will combine tissue culture techniques, immunological assays, and gene expression studies.

## Prof Steve Gerondakis and A/ Prof Gilda Tachedjian

Two vacancies

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A family of cell surface and intracellular proteins, the so-called Toll-Like Receptors (TLRs), coordinate the innate immune response to microbial pathogens by recognizing specific microbial products such as bacterial cell wall components or double stranded RNA. Understanding how different microbial molecules invoke specific patterns of gene expression underscore our understanding of normal innate immunity and immune associated pathology. Our laboratory focuses on the roles of the Nuclear Factor of Kappa B (NF $\kappa$ B) and Mitogen-Activated Protein Kinase (MAPK) pathways in controlling gene expression in response to TLR signalling. The two projects outlined below investigate distinct aspects of the roles one or both pathways serve in innate immune responses.

## **Understanding how cells protect themselves from the cytotoxic compounds produced during innate immune responses.**

**(Prof Steve Gerondakis, A/Prof Gilda Tachedjian & Dr Ashish Banerjee)**

The innate immune response initiated by immune receptors, including TLRs, involve the production of anti-microbial and cytotoxic compounds by cells such as macrophages, a number of which are also potentially deleterious to the immune cells that produce them. Consequently, immune cells have developed a range of mechanisms to protect themselves from damage or death following the synthesis of toxic anti-microbial factors such as TNF, Type I interferons and free radicals. Surprisingly little is known about the signalling pathways that control these protective responses to cytotoxic immune regulators. We have shown that the NF $\kappa$ B pathway is critical in controlling the protective responses to TNF and interferon in macrophages following activation of TLR3 and TLR4, receptors typically activated during viral and bacterial immune responses respectively. This project will involve the use of genetics, cell biology and molecular biology to ascertain which specific signalling molecules downstream of the NF $\kappa$ B pathway are required to protect TLR3 activated macrophages from TNF and Interferon induced cell death.

## **Coordinating gene expression during innate immune responses.**

**(Prof Steve Gerondakis, A/Prof Gilda Tachedjian & Dr Ashish Banerjee)**

Patterns of gene expression induced by specific microbial products such as double stranded RNA or bacterial cell wall components reflect the different combination of transcription factors activated by the signalling pathways located downstream of particular TLRs. Amongst the intracellular signalling pathways engaged by TLR4, the receptor for lipopolysaccharide (LPS), are the Extracellular Regulated Kinase (ERK) and NF $\kappa$ B pathways. Notably, many of the genes induced or repressed in LPS activated macrophages are co-regulated by ERK and NF $\kappa$ B. The importance this combination of signalling pathways serves in controlling TLR4 dependent gene expression is underscored by the finding that the activation of both is coordinated by a cytoplasmic complex comprising the NF $\kappa$ B1 transcription factor and the Tpl2 kinase, a master regulator of ERK activation. Understanding how this signalling complex coordinates NF $\kappa$ B and ERK dependent gene expression has important implications for the treatment of pathology arising from microbial infections and inflammation. Using a combination of cell culture methods, microarray and Real-time PCR analysis of gene expression plus Chip analysis of transcription factor binding to gene promoters, this project aims to understand how NF $\kappa$ B and ERK coordinate the transcription of specific genes in TLR4 activated macrophages.

### **Dr Heidi Drummer**

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## **Epitope shielding by the variable regions of Hepatitis C Virus glycoprotein E2.**

Hepatitis C Virus infects 3% of the world's population and is the leading indicator for liver transplantation in Western countries. Currently there is no vaccine to prevent infection and

therapy is limited to the use of pegylated interferon and ribavirin with limited efficacy. The HCV glycoproteins E1 and E2 form heterodimers and mediate attachment of virions to cells and membrane fusion. Understanding how these proteins operate during viral entry is essential for the development of vaccines and antiviral agents to prevent and cure infection. Glycoprotein E2 mediates binding to cellular receptors and is also a major target for neutralizing antibodies. Glycoprotein E2 comprises three variable regions that alternate with conserved regions encompassing the receptor binding site and major neutralization epitopes. The variable regions form surface exposed flexible structures and we propose that they shield the underlying core domain from neutralizing antibody. This project will examine how the size, sequence and glycosylation of variable regions modulates the efficacy of neutralizing antibodies.

This project will require work in both PC2 and PC3 laboratories following training.

### **Dr Andy Pombourios**

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### **Mechanisms for receptor-mediated activation of the HIV-1 envelope glycoprotein complex.**

The envelope glycoprotein complex of HIV-1 comprises a receptor binding glycoprotein, gp120, in association with a fusion glycoprotein, gp41. gp120-receptor interactions trigger structural changes in gp41 that cause fusion of the virus and cell membranes. These events lead to the formation of a fusion pore, an aqueous channel through which the viral genome penetrates the cytosol of CD4<sup>+</sup> T cells, macrophages, dendritic cells and microglia. In this project, forced sequence evolution will be used to map regions in gp120 and gp41 that contribute to the activation of membrane fusion function. Replication defective viruses with mutations in the gp120-gp41 association site will be serially passaged in primary T cells and T cell lines in order to select revertants with 2nd and 3rd site mutations that restore replication competence. The location of 2nd and 3rd site mutations will be identified by DNA sequencing and the mechanism by which functionality is restored determined using biochemical and cell biological approaches. The overall goal of the project is to understand how the gp120-gp41 complex is activated by receptor and to identify new conserved targets for fusion inhibitor development.

This project will require work in both PC2 and PC3 laboratories following training.

## Victorian Infectious Diseases Reference Laboratory (VIDRL)

### Dr Peter Reville

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One vacancy

Hepatitis B virus is one of the most important human pathogens. Over 400 million people currently have chronic HBV infection, resulting in up to 2 million deaths annually. Although a DNA virus, an RNA pre-genome is critical in HBV replication. Importantly, numerous spliced variants have been identified in persons with chronic HBV infection, one of which (Sp1) encodes a novel protein- the hepatitis B splice protein (HBSP). Although these spliced variants are incapable of autonomous replication, they are always present in association with wild-type (wt) virus, which rescues their replication. It has recently emerged that the presence of a spliced variant (Sp10) leads to an increase in wt HBV replication, for reasons that are unclear. The effect of the major Sp1 spliced variant on HBV replication is unclear. This project will compare the effect of the Sp1 and Sp10 spliced variants on HBV replication and determine where in the HBV life cycle an effect on HBV replication is manifested. A large range of molecular techniques will be employed in this project, including PCR, cloning, cell culture, transfection, and Southern/Northern/Western blotting.

**MONASH MICROBIOLOGY HONOURS 2010**

Name:

Address in December/January:

Mobile Phone (9am-5pm):

Phone (Home):

Email (must be checked daily):

**Project preferences (supervisor and brief project title):**

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You **must** have talked to three potential supervisors before submitting this form.

Supervisor 1 Signature: \_\_\_\_\_  
Supervisor 2 Signature: \_\_\_\_\_  
Supervisor 3 Signature: \_\_\_\_\_

Please return this form to Professor Julian Rood as soon as possible, but no later than November 23, 2009.