



Medicine, Nursing and Health Sciences

Honours Programs in Microbiology 2012

Department of Microbiology



2012 Honours Coordinator

Professor Julian Rood

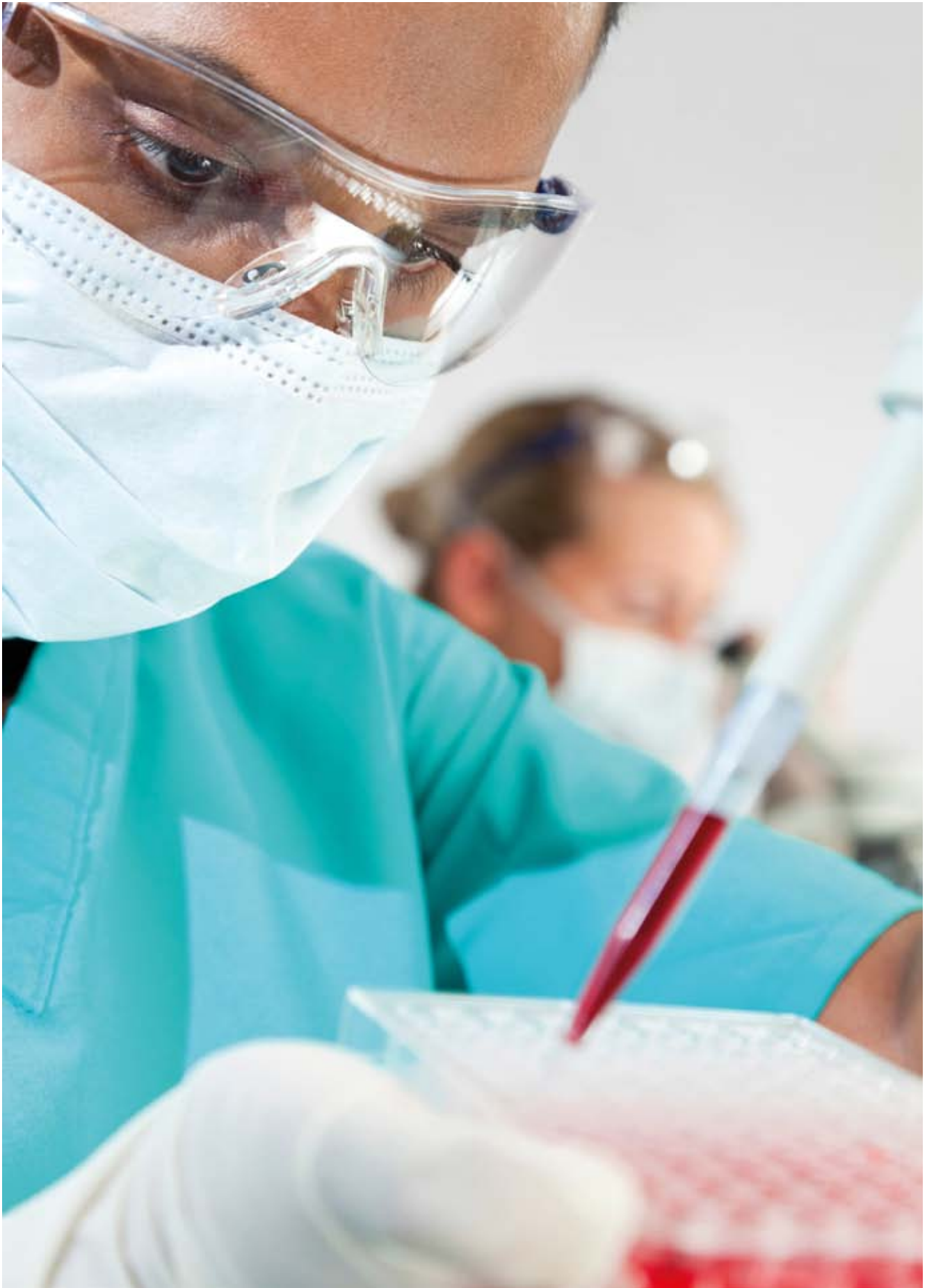
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2012 Honours Programs in Microbiology

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 2012. 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 18.
B. Sc (Hons):
www.sci.monash.edu.au/undergrad/honours/apply.html
B. Biomed. Sci (Hons):
www.med.monash.edu.au/biomed/honours/
2. Submission of project preferences to Professor Julian Rood (no later than November 25, 2011).

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 2012 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 Professor 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 25 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, and may also include a statistics course and a seminar series. BSc students need to complete two colloquia, which together with the statistics course and the seminar series comprise the unit MIC4200: Advanced Studies in Microbiology, and BMS students one colloquium, in addition to other microbiology-based coursework and 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 20, 2012 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% |
| Microbiology coursework | 25% |

Final assessment of the BMS Honours program follows the format:

| | |
|-------------------------------|------|
| Literature survey | 7.5% |
| Research report/report review | 60% |
| Seminar | 7.5% |
| Microbiology coursework | 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 per cent 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 per cent in their remaining level-three units. Alternatively, such students may be permitted to enrol in a Master of Biomedical Science (Part 1) 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 per cent 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 per cent 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 2012



Professor Julian Rood

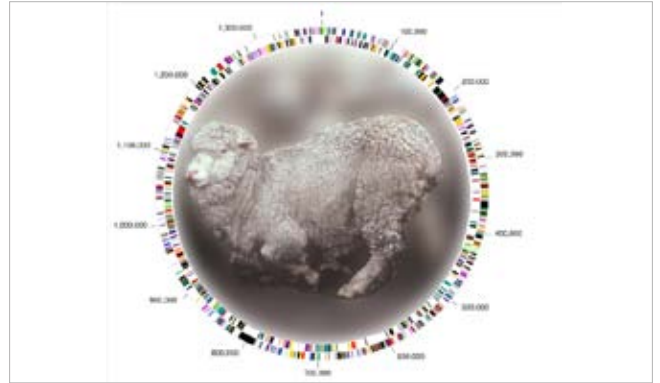
Two vacancies

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Xiaoyan Han, Trudi Bannam, Ruth Kennan, Jackie Cheung, Luminita Badea, Julian Rood, Versha Rai



D. nodosus genome – ovine footrot

www.med.monash.edu.au/microbiology/research/rood.html

Functional Biology of Bacterial Pathogens

Mechanism of plasmid replication in *Clostridium perfringens*

Dr Trudi Bannam and Professor 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 analysis of putative chitinases from a necrotic enteritis strain of *Clostridium perfringens*

Dr Xiaoyan Han and Professor Julian Rood

Avian necrotic enteritis is an economically important enteric disease that is caused by specific strains of *Clostridium perfringens* type A. We have recently discovered a novel pore-forming toxin, NetB, which is essential for virulence. The netB structural gene is carried on a conjugative plasmid that encodes several other potential virulence factors, including two putative chitinases. This project aims to construct the mutants in these two potential chitinase genes and to determine their function by analysing the phenotypic effect of these mutations.

Functional characterization of a putative bacitracin resistance cassette from *Clostridium perfringens*

Dr Xiaoyan Han, Dr Trudi Bannam and Professor Julian Rood

Bacitracins are a mixture of structurally related cyclic polypeptides with clinically useful antibiotic properties. They act by indirectly interfering with the biosynthesis of the bacterial cell wall. We recently discovered an avian necrotic enteritis strain of *Clostridium perfringens* that was resistant to bacitracin and identified, for the first time in this genus, a putative bacitracin resistance locus containing the potential structural genes *bacrABD* and a regulation gene *bcrR*. To determine if this locus is responsible for bacitracin resistance, this project will determine which genes are essential for this antibiotic resistance phenotype and how the expression of this potential ABC transporter is regulated. This study will involve the construction of individual mutants in each gene of the bacitracin resistance locus. Subsequently, the effect of these mutations on bacitracin resistance and gene expression will be characterized. The successful completion of this project will provide important functional information on this novel antibiotic-resistant determinant.

How does *C. difficile* regulate the production of binary toxin?

Dr Glen Carter, Dr Dena Lyras and Professor 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.

Regulation of extracellular toxin production in *Clostridium perfringens*

Dr Jackie Cheung and Professor Julian Rood

Clostridium perfringens is the causative agent of gas gangrene and food poisoning and produces many different toxins and extracellular enzymes. Studies in this laboratory have shown that the production of several of these toxins is regulated by the VirS/R two-component signal transduction system. Using a gas gangrene strain of *C. perfringens* we have recently identified a new signal transduction system that involves the orphan response regulator RevR, which regulates the production of several potential virulence factors as well as the expression of genes involved in phosphate homeostasis. DNA binding studies show that RevR binds to DNA sequences called Pho boxes that are located upstream of the phosphate homeostasis genes. The aim of this project is to expand our knowledge of RevR by determining the role of this system in a food poisoning isolate of *C. perfringens*. This project will involve the use of molecular biology, microbial genetics and transcriptomic analysis to determine the functional role of RevR in the regulation of virulence genes in *C. perfringens*.

Clostridium-directed enzyme prodrug therapy (CDEPT) – Helping ‘bugs’ to cure cancer: identification of novel NTR proteins with improved prodrug converting properties

Dr Luminita Badea, Dr Glen Carter and Professor Julian Rood

According to the World Health Organization (WHO) an approximately 12 million people worldwide will die of cancer in 2030. Although some cancers can be treated, many malignancies, including those associated with solid tumours, remain difficult to cure. Most treatments result in inefficient targeting of therapeutic agents to solid lesions and rely on methods that often are associated with adverse side effects. Directed enzyme prodrug therapies (DEPT) rely on the deliveries of the enzymes (such as nitroreductase, NTR, or cytosine deaminase, CD) that convert inactive drugs, known as prodrugs, to highly active anti-cancer agents specifically within the tumour mass. Selective delivery of these enzymes ensures that the prodrug is converted to the active form only within the tumour and reduces the exposure of healthy tissue to the toxic effect of anti-cancer agent, reducing side-effects. *Clostridium*-directed enzyme prodrug therapy (CDEPT) utilizes the spores of recombinant clostridial species that

have been genetically engineered to produce prodrug converting enzymes. This therapy offers strict tumour specificity because clostridia only germinate in the hypoxic centre of solid tumours. Presently, much work is focused on increasing the efficacy of the prodrug converting enzymes. Initially, several strong promoters used to drive expression of NTR or CD in clostridia have been identified. In addition to increasing expression levels of NTR and CD, we are interested in finding novel NTR and CD proteins with improved prodrug converting properties. The objective of this project is to identify novel nitroreductase (NTR) proteins with improved prodrug converting properties. This project will use general molecular biology techniques, gene cloning, PCR, protein expression, enzyme assays, Western blotting and cell cytotoxicity assays.

Genetics of toxin plasmids of *Clostridium perfringens*

Dr Vicki Adams and Professor 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, D and E strains of *C. perfringens* are conjugative and to determine their genetic organisation.

Glycosylation of *Dichelobacter nodosus* fimbriae

Dr Ruth Kennan and Professor Julian Rood

In recent years there have been increasing reports of the glycosylation of surface proteins of various gram-negative bacteria, including many important pathogens. These glycosyl residues are often found on proteins that are important in pathogenesis, including pili (fimbriae), adhesins and flagella. The type IV fimbriae of some *Neisseria* and *Pseudomonas* species are glycosylated, and there is evidence that this glycosylation may play a role in adhesion and twitching motility. *Dichelobacter nodosus* is the principle causative agent of footrot, a highly contagious and economically significant bacterial disease affecting sheep in most countries. In *D. nodosus*, type IV fimbriae are essential for twitching motility and virulence and appear to be involved in protease secretion. There is limited evidence that the fimbriae of some *D. nodosus* strains may be glycosylated, but the possible functional role of glycosylation has not been investigated. The overall aim of this project to investigate the glycosylation of the *D. nodosus* type IV fimbriae by screening several strains for evidence of fimbrial glycosylation. Chromosomal mutants will also be constructed in sugar transport genes, and the effect of these mutations on fimbriae glycosylation examined and the phenotypic properties of those fimbriae will be investigated.

Gene regulation in *Dichelobacter nodosus*

Dr Ruth Kennan and Professor 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. *D. nodosus* has a small genome of 1.4 Mb, which has been sequenced. Only a small number of regulatory genes were identified, including four two-component regulatory systems, two of which are involved in the regulation of the fimbriae. The overall aim of this project is to construct chromosomal mutants in the other two-component systems and to identify genes that are up- and down-regulated by these systems by comparing the transcriptomes of the wild-type and the mutants.

The survival of *Clostridium perfringens* in infected tissues

Dr Milena Awad, Dr Dena Lyras and Professor Julian Rood

Clostridium perfringens is the causative agent of clostridial myonecrosis or gas gangrene and is an aerotolerant anaerobe that does not grow in the presence of oxygen. The ability of vegetative *C. perfringens* cells to survive their initial exposure to oxidative stress in the tissues is important in the early stages of infection and the presence of a localised anaerobic environment, usually as a result of trauma-induced tissue ischaemia,

is essential for the establishment of a *C. perfringens* infection and the subsequent development of myonecrotic disease. Reactive oxygen species, such as superoxide and hydrogen peroxide, cause oxidative stress in bacteria. Superoxide dismutase, catalase and peroxidases are involved in the detoxification of reactive oxygen species in some anaerobes. Although *C. perfringens* is catalase negative, analysis of the genome sequence reveals the presence of several genes that encode putative enzymes that may contribute to resistance to oxidative stress. These enzymes include a superoxide dismutase, two glutathione peroxidases, a putative alkyl hydroperoxide reductase and two methionine sulfoxide reductases. The objective of this project is to determine the role of one or more of these genes in the response of *C. perfringens* to oxidative stress and its ability to grow *in vivo* and cause disease. It will involve the construction and complementation of chromosomal mutants, their subsequent *in vitro* analysis and virulence testing in a mouse myonecrosis model.

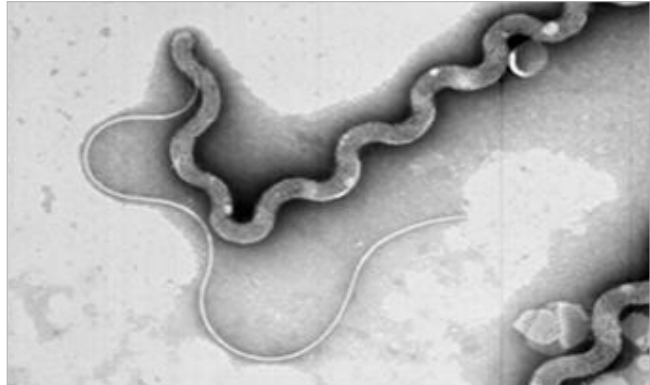


Professor Ben Adler

Up to two vacancies
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The Adler and Boyce Groups



EM of *Leptospira*

www.med.monash.edu.au/microbiology/staff/adler/adlerhp.html | www.microbialgenomics.net

Pathogenesis and molecular biology of leptospirosis

Dr Gerald Murray, Dr Renée Marcsisin and Professor Ben Adler

Leptospira spp. are responsible for the most widespread zoonosis in the world, as well as being a cause of disease in production and companion animals. 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 characterisation of *Leptospira* mutants generated in our laboratory using a recently developed transposon mutagenesis method. Current areas of interest include: development of vaccines against leptospirosis; transcriptomics 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; genes involved in colonisation of the kidney tubules. The results of these studies will contribute to the understanding of leptospiral pathogenesis, and help to attribute function to the ~40 per cent of leptospiral genes that have no assigned function.

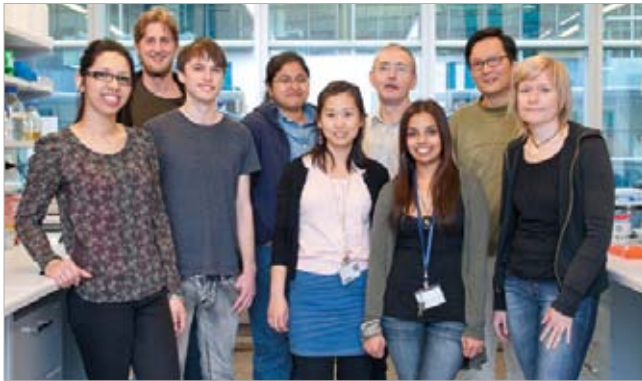
Pasteurella multocida: defining the mechanisms of pathogenesis and immunity

Dr Marina Harper, Professor Ben Adler and Dr John Boyce

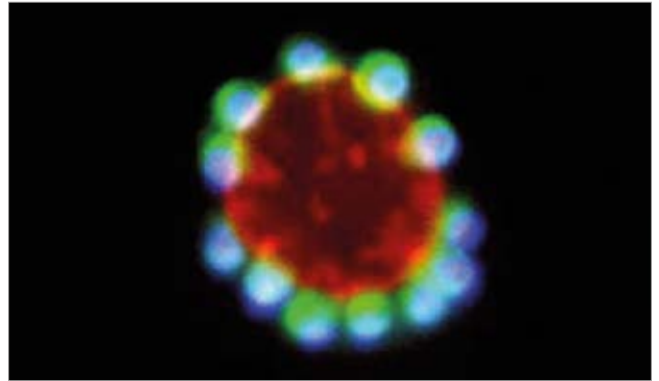
Pasteurella multocida is a Gram-negative bacterial pathogen that causes a number of different diseases in cattle, pigs and poultry, resulting in serious economic losses worldwide in food production industries. We are interested in understanding the molecular mechanisms of pathogenesis in this bacterium 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. *Pasteurella multocida* expresses a number of adhesins and outer membrane proteins, but the precise role of some of them in the pathogenesis of disease has not been determined. This project will use Targetron mutagenesis to inactivate selected surface proteins and test the effect of these mutations on strain phenotype and/or virulence. These experiments will help to elucidate the role of surface proteins in disease caused by *P. multocida*.

Professor Brian M Cooke

Three vacancies
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The Cooke lab



Malaria parasites invading a RBC

Haemoprotozoan 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 experienced researchers in the laboratory. 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.

Characterisation of malaria PHIST-domain proteins

Dr Nicholas Proellocks and Professor Brian M Cooke

Proteins containing a PHIST (*Plasmodium* helical interspersed sub-telomeric) domain constitute a multi-member family that are present in the most important species of human malaria parasites. Although the family is largely uncharacterised, preliminary information on the function of a few characterised members suggest that they play major roles in pathogenesis. This project will use a combination of molecular, cellular, proteomic and biophysical approaches to characterise defined members of PHIST proteins to gain a better understanding of the mechanisms by which malaria parasites cause human disease. This will involve both *in vitro* studies of protein interaction and *ex vivo* studies of protein fate and action in transgenic parasites. Specifically, your studies will contribute to an overall analysis of the expression and cellular localisation of previously uncharacterised PHISTs, determine their function in PRBCs and identify the host and/or parasite proteins with which they interact.

Understanding the function of unique *P. falciparum* FIKK kinases

Dr Nicholas Proellocks, Dr Svenja Günther and Professor Brian M Cooke (in collaboration with Professor Christian Doerig)

FIKK kinases are unique among apicomplexan parasites. Interestingly the genome of *P. falciparum* encodes 20 FIKK kinases, but very little is known about the localisation of biological function of any of these. This project will focus on a sub-group of FIKK kinases which our preliminary work strongly suggests are essential for parasite survival. The divergence of these enzymes from mammalian eukaryotic protein kinases, together with their essentiality to parasite proliferation, makes them highly attractive potential targets for the development of next-generation anti-malarial drugs. The overall objective is to characterise one or

more essential FIKK kinases and help to understand the mechanisms by which they regulate essential biological processes in the parasite. This will be achieved in part by confirming their essentiality, localisation and trafficking in blood stage *P. falciparum* parasites and identification of their target proteins.

Function of skeleton binding protein 1 (SBP1) in malaria-infected red blood cells

Professor Brian M Cooke and Donna Buckingham

We have previously characterised an exported protein in malaria-infected RBCs (called SBP1) that is essential for trafficking and translocation of a major virulence protein (PfEMP1) onto the surface of infected RBC. Clearly, this protein is central to the pathogenicity of malaria parasites, however, its precise mechanism of action still remains unknown. We know that SBP1 binds directly to the membrane skeleton of RBCs but the specific proteins with which it interacts (and sub-domains within them) have not yet been identified. In this project we will undertake a complete characterisation of the interaction between the C-terminal domain of SBP1 and specific proteins of the RBC membrane skeleton. Using pull-down assays and a panel of recombinant proteins, we will define the minimal binding domains of each interacting partner and quantify the binding affinities of these interactions using Surface Plasmon Resonance (SPR). We will also examine the ability of a series of SBP1 truncation, deletion and substitution mutants to complement the PfEMP1 trafficking defect in existing *sbp1* knockout parasites. Overall, the project will help to get a better understanding of the mechanisms by which malaria parasites transport proteins beyond the confines of their own cell membrane and may ultimately inform the future design of selective and potent new anti-malarial drugs.

Studies on babesia

Babesia bovis is an important haemoprotozoan 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 of 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.

The life cycle of *Babesia bovis*

Dr Svenja Günther, Sejal Gohil and Professor Brian M Cooke

The first step in better understanding this parasite is to understand its life cycle. Currently, we do not know how long it takes to progress through the intraerythrocytic cycle and how the different developmental stages of the parasite are defined. The aim of this project is to investigate in detail

the parasites life cycle in red blood cells by live cell microscopy to elucidate precisely how long it takes for the parasite to develop. In addition, we will analyse specific parasite organelles to gain insight into their development as well as using them as markers for the different life cycle stages. To achieve this, this project will involve the generation of mutant *B. bovis* parasite lines expressing and targeting green fluorescent protein to different organelles. Mutant parasite lines will then be analysed by live cell microscopy. In addition, organelle specific antibodies will be employed as tools for visualising the different developmental events in *B. bovis*.

Characterisation of novel *Babesia bovis* exported parasite proteins

Dr Svenja Günther, Sejal Gohil and Professor Brian M Cooke

The mechanisms by which *Babesia bovis* causes severe disease in susceptible cattle are not well understood, however, it is clear that alterations to the structure and function of infected RBCs, secondary to the export of currently uncharacterised parasite-encoded proteins play a critical role. Using a rational bioinformatic analysis of the recently annotated genome sequence of *B. bovis*, we have identified a subset of parasite proteins that we predict will be exported from the parasite into the host RBC and play a major role in host cell modification. The overall aim of this project is to characterise these novel exported proteins by determining their localisation within the infected RBC and ultimately elucidating their function. A combination of bioinformatic, molecular, cellular and proteomic approaches will be employed in order to shed some light on the mechanisms by which these parasites induce dramatic changes to the infected RBC. A complete analysis of the parasites' 'exportome' will result in a better understanding of the pathogenesis of babesiosis and facilitate the identification of new therapeutic strategies to combat this disease.

Identification of the *Babesia bovis* ridge protein

Dr Svenja Günther, Sejal Gohil and Professor Brian M Cooke

Shortly following invasion, *Babesia bovis* induces the formation of unique structures on the RBC surface, which we have termed 'ridges'. Importantly, the appearance of ridges appears to correlate with the level of parasite virulence and are believed to be the structures responsible for the binding of infected RBC to endothelial cells therefore mediating the 'vasculature-blocking' phenotype of the parasite. The protein(s) responsible for the formation of these ridges on the cell surface currently remain unknown. In this project we will use a combination of molecular, biochemical and proteomic approaches to identify the 'ridge' protein. Elucidation of this protein and ultimately its function in the infected RBC will give us a better understanding of the pathogenesis of bovine babesiosis.

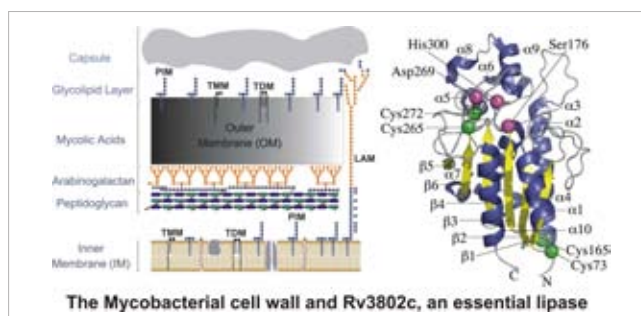
Professor Ross Coppel



Professor Ross Coppel and Dr Paul Crellin

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www.med.monash.edu.au/microbiology/research/coppel/

Studies on Tuberculosis

Dr Paul Crellin and Professor Ross Coppel

Tuberculosis (TB) is the leading cause of death in the world from a single infectious agent. 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 re-emerging. *M. tuberculosis* is well adapted to survival within its human host and can resist the bactericidal actions of the immune system. The bacterium is naturally resistant to many antibiotics and now, in addition, many strains have acquired resistance to drugs used specifically to treat TB patients. The recent emergence of totally drug resistant (TDR-TB) strains is of great concern and highlights the urgent need for new therapeutic strategies. Mycobacteria are able to withstand immunological and chemical attack partly because they have very robust cell walls. This unusual, multilayered structure is essential to mycobacterial growth and survival and the target of many existing TB drugs. We are studying the genetics and biochemistry of mycobacteria with the ambition of developing new drugs to combat this devastating disease.

Targeted mutation of cell wall genes

Using bioinformatics analyses of the *M. tuberculosis* genome, we will identify genes likely to be involved in the biosynthesis of particular mycobacterial cell wall components. These genes will be disrupted using allelic exchange methods and the resultant mutant bacteria characterized for changes in the cell wall. Since cell

wall biosynthesis enzymes tend to be essential for the growth and survival of mycobacteria, the mutants will be made in a related species, *Corynebacterium glutamicum*, which is less sensitive to cell wall lesions and has proven to be an informative model for studying mycobacterial cell wall biosynthesis.

Expression of potential drug targets

Enzymes that are essential for mycobacterial cell wall biosynthesis are potential targets for intervention by new generations of antibiotics. We will clone, express and purify these enzymes from the model species *Mycobacterium smegmatis* for enzymatic studies, crystallisation, structural determination, and future rational drug design experiments. Of particular interest are: (1) Pks13, a massive polyketide synthase responsible for a key step of cell wall mycolic acid biosynthesis, (2) CmrA, a reductase that produces mature mycolic acids, and (3) Rv3802c, an essential cell wall lipase inhibited by the human obesity drug tetrahydrolipstatin.

Identification of protein complexes that regulate cell wall synthesis

Recent studies indicate that the regulation of mycobacterial cell wall synthesis is more complicated than first thought and involves multiple, interacting proteins working in concert. We will determine whether cell wall biosynthesis proteins previously identified in our laboratory participate in such complexes using techniques including two-hybrid screening, protein crosslinking and pull-down experiments.

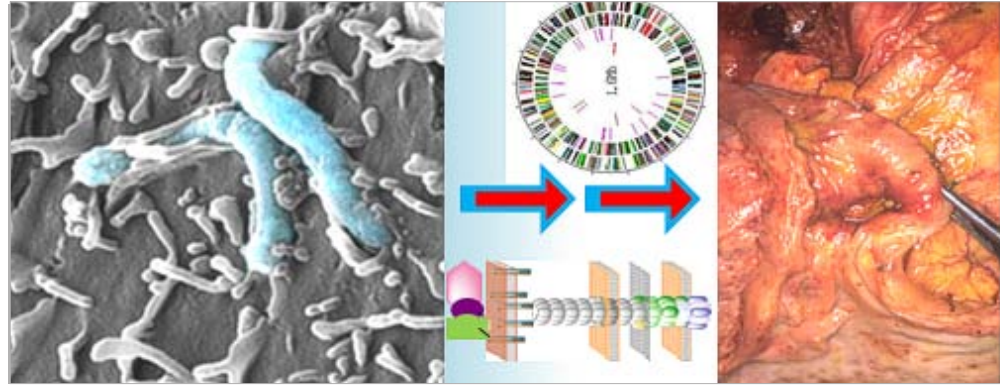
Dr Terry Kwok-Schuelein

Two vacancies

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Dr Terry Kwok-Schuelein

Unravelling the molecular mechanisms by which *Helicobacter* causes cancer

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 (NFκB) 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.

Dr Dena Lyras



Dr Dena Lyras

How do the clostridia control the production and export of the large clostridial toxins?

Dr Glen Carter and Dr Dena Lyras

The large clostridial toxins (LCTs) are an important family of bacterial virulence factors that includes toxin A (TcdA) and toxin B (TcdB) from *Clostridium difficile*, lethal toxin (TcsL) and haemorrhagic toxin (TcsH) from *Clostridium sordellii*, alpha toxin (TcnA) from *Clostridium novyi* and TpeL from *Clostridium perfringens* type C strains. The LCT producing clostridia are important human and animal pathogens. *C. difficile* for example is regarded as a hospital 'superbug' and is estimated to cost the US healthcare system \$3.2 billion per year. *C. sordellii* causes severe disease in heroin users and in women undergoing medically induced abortions, resulting in extremely high mortality rates that exceed 70 per cent. Similarly, infection with *C. novyi* causes a debilitating disease which results in the death of approximately 50 per cent of patients. *C. perfringens* type C strains cause necrotic enteritis, an often fatal disease characterised by intestinal necrosis and septicemia. Despite their obvious impact on public and veterinary health, the disease pathogenesis of the LCT producing clostridia is poorly understood, and little is known about the expression or secretion of these toxins. Bioinformatic analysis has led us to identify loci within each organism that we hypothesise control both the expression and export of these toxins by a conserved mechanism. Using a molecular genetic approach we will determine the role that the genes within these loci play in toxin production and export, and in disease pathogenesis of the LCT producing clostridia. Determining the function of these genes may provide novel therapeutic targets which can be exploited to control the debilitating diseases caused by these important human and animal pathogens.

Antibiotic resistance, virulence and mobile genetic elements in *Clostridium sordellii*

Dr Milena Awad and Dr Dena Lyras

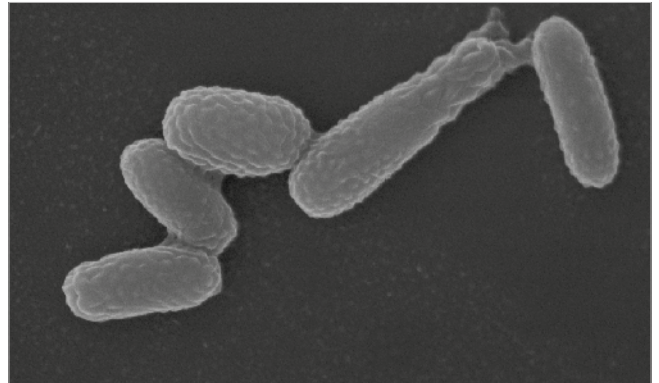
Clostridium sordellii is an emerging human pathogen that causes rapidly progressing tissue necrosis, shock, a characteristic immune response and multi-organ failure. It has a very high mortality rate of approximately 70 per cent, reaching 100 per cent for postpartum patients, and has been associated with infections following spontaneous

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Scanning EM of *C. difficile* spores

or medically induced abortion, notably following the administration of mifepristone (RU486). Little is known about how *C. sordellii* causes disease, however toxin production appears to play a major role. We have now developed methods for carrying out genetic analysis in *C. sordellii* making it possible to use molecular approaches to genetically manipulate this organism. Numerous mobile genetic elements have been found to encode antimicrobial resistance and virulence genes in other clostridial species but very little is known about the presence of these elements in *C. sordellii*. Similarly antibiotic resistance in this organism has not clearly been defined. The focus of this project will be to establish both toxin and antibiotic resistance profiles of human and animal *C. sordellii* strains, to determine if they are located on mobile genetic elements and to genotypically and phenotypically characterise these elements.

Understanding the host immune response to *Clostridium difficile* infection

Dr Glen Carter, Ms Anjana Chakravorty and Dr Dena Lyras

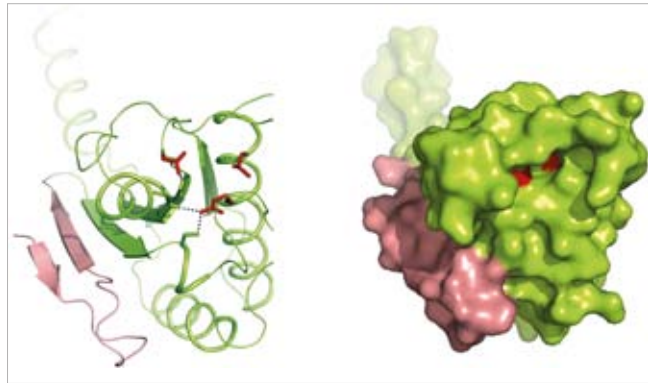
Clostridium difficile is recognised as the major cause of nosocomial diarrhoea in Australian hospitals and in hospitals worldwide. Chronic colitis syndromes caused by this organism are a significant cause of morbidity in hospitals with control and treatment costs rapidly escalating. The recent emergence 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 numerous toxins and other virulence factors, however, there is considerable genetic variation between disease-causing strains in this regard. We have recently developed a mouse model of infection which closely mimics human infection. This project will use the mouse model of *C. difficile* infection to assess the host immune response to *C. difficile* infection, in particular using specific mutants of clinically relevant *C. difficile* strains. Our primary focus will be on the ability of *C. difficile* toxins to modulate the cytokine response during disease and we will extend these studies into a broader exploration of the pathways involved downstream of these responses.

Associate Professor
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Associate Professor Anna Roujeinikova



H. pylori motility protein B

Structural biology of *Helicobacter pylori* virulence factors

Helicobacter pylori and gastric cancer: the role and the mechanism of CagA

Associate Professor A Roujeinikova and Dr T Kwok-Schuelein

Pathogenic strains of *Helicobacter pylori*, associated with the development of adenocarcinoma in humans, inject CagA protein into gastric epithelial cells, where it interacts with many different host cell proteins, interfering with signalling pathways that regulate the cell growth and motility. Detailed characterization of the CagA structure and interactions will be undertaken for elucidation of its role in gastric carcinogenesis. In complementing experiments, the *in-vivo* activity of CagA fragments (e.g. effect on cell morphology and motility, interaction with various partner molecules) will be assessed. We aim to isolate and analyze stable CagA domains suitable for structural studies and identify the smallest CagA fragment required for translocation and initiation of transformation of gastric epithelial cells.

Bacterial flagellar motor: structure and dynamics of *Helicobacter pylori* motility protein B

Associate Professor A Roujeinikova and Associate Professor A Buckle

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 initial stomach colonization by *Helicobacter pylori* and for attaining full infection levels. Bacterial motility, and the MotB function in particular, can be used as an unconventional antibacterial target to cure or prevent disease. 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. To understand how the power-generating system assembles and functions, we will characterise MotB dynamics using a combination of X-ray crystallography, small-angle X-ray scattering, fluorescent spectroscopy and NMR.

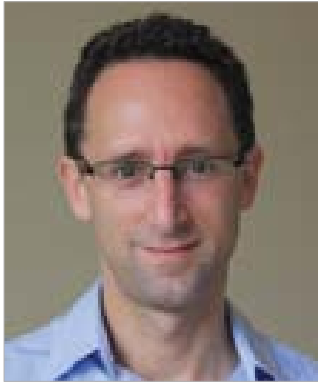
Dr Anton Peleg

One vacancy

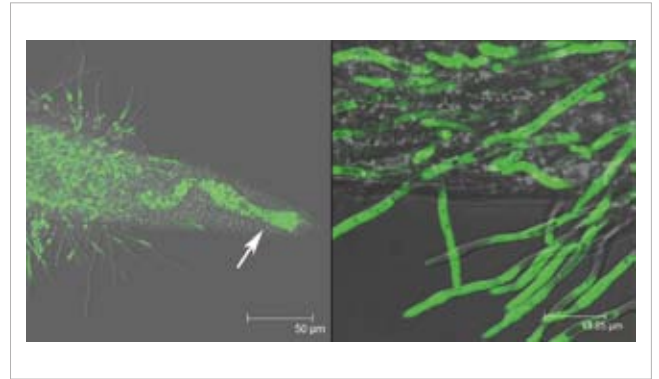
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Dr Anton Peleg



Candida infected worms

The study of *Candida albicans* filamentation and virulence using a *Caenorhabditis elegans* model

Dr Anton Peleg and Dr Ana Traven (Department of Biochemistry and Molecular Biology)

Candida albicans is the most common human fungal pathogen, causing life-threatening, invasive disease in the most vulnerable patients, including those suffering from cancer, HIV, diabetes, heart disease and those undergoing major surgery and organ transplantation. *Candida* has become the fourth leading cause of hospital-acquired bloodstream infection, and invasive disease is associated with a mortality of ~40 per cent. Key virulence determinants of *C. albicans* toward humans are: (i) the ability to grow with an elongated, filamentous cell morphology, and (ii) the ability to form drug-resistant biofilms, particularly on implanted medical devices. A detailed understanding of the mechanisms controlling biofilms and filamentous growth would represent a significant breakthrough in the development of desperately needed therapeutic strategies against this troublesome human pathogen.

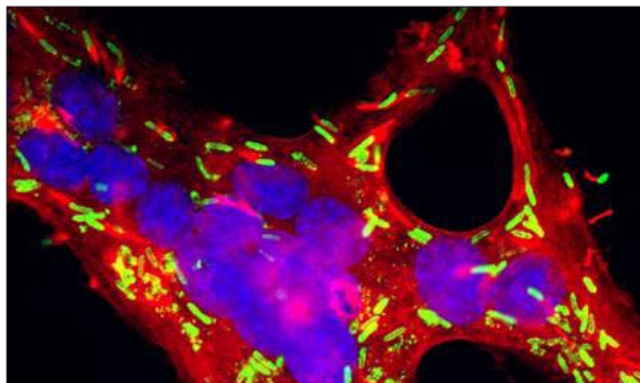
We have developed an *in vivo* assay using an invertebrate worm model (*C. elegans*) to study *Candida* filamentation and virulence. We have screened a *Candida* mutant library and identified several novel genes that may be important for *Candida* filamentation and virulence. The aim of this project is to characterize one of these genes by constructing a targeted mutant and complemented strain, and to assess its significance to *Candida* virulence using a range of *in vitro* (filamentation, biofilm formation, antifungal susceptibility) and *in vivo* assays (*C. elegans* and mammalian model). This project will be co-supervised by Dr Anton Peleg (Dept of Microbiology) and Dr Ana Traven (Dept. of Biochemistry) who have unique skills in the virulence assessment and genetic manipulation of *Candida albicans*.

Dr John Boyce

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Dr John Boyce



Macrophages infected with *Burkholderia*

www.med.monash.edu.au/microbiology/staff/boyce/boycehp.html

Understanding virulence mechanisms of *Burkholderia pseudomallei* for improved treatment options and rational vaccine development

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. *B. pseudomallei* has recently been classified as a category B bioterrorism agent and is believed to have the potential to be used as a biological weapon. No vaccine for melioidosis exists and little is known about the molecular mechanisms of *B. pseudomallei* virulence. The organism is intrinsically resistant to numerous antimicrobial agents and infections progress rapidly resulting in reduced treatment options. Despite much research on prevention and treatment, mortality rates remain high (up to 90 per cent). Consequently, novel treatment options are urgently needed. Therefore, we are interested in identifying novel bacterial virulence factors that may be candidate vaccine antigens or targets for anti-infective drugs.

Characterisation of two-component signal transduction systems in *B. pseudomallei* and their potential use as targets for anti-infective drugs

Dr Elizabeth Allwood, Professor Ben Adler and Dr John Boyce

Two-component signal transduction systems (TCSTS) allow bacteria to sense and respond to changes in environmental conditions and are therefore important regulators of bacterial gene expression. *B. pseudomallei* has over 60 TCSTS; however, only three have been studied thus far. We recently identified the first virulence-associated TCSTS in *B. pseudomallei* which regulates flagellin expression and controls motility and virulence. This study will characterise other TCSTS in *B. pseudomallei*. A range of techniques will be utilised including directed mutagenesis, virulence trials using a murine melioidosis model and numerous assays for phenotypic characterisation of the constructed mutants. This study also has scope for whole-genome transcriptomics using RNA sequencing, to determine the genes regulated by

specific TCSTS. This will in turn provide a guide as to the most appropriate phenotypic assays for functional characterisation of the mutants. Importantly, TCSTS could regulate a large array of virulence-associated genes. As such, they make attractive targets for anti-infective drugs which would ultimately provide an alternative treatment option for melioidosis where few currently exist.

Antibiotic resistance 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 including polymyxin antibiotics such as colistin. As a consequence *A. baumannii* is recognised as one of the six top-priority MDR pathogens worldwide.

Understanding mechanisms of colistin resistance in *A. baumannii*

Dr Marina Harper, Dr Rebekah Henry and Dr John Boyce

Acinetobacter baumannii has become resistant to almost all current antibiotics. Colistin is now used as a last-line therapy against MDR *A. baumannii*, but infections caused by colistin-resistant strains are an emerging problem. We have recently received a paired set of colistin-sensitive and colistin-resistant clinical isolates recovered from a patient that was treated with colistin. Initial comparison of the genomes of these two strains indicates that the acquisition of colistin resistance is associated with the movement of a mobile insertion sequence (IS) element. This project will initially identify the positions of the IS elements in the genome of each strain and then use a range of mutagenesis, complementation and transcriptomic approaches to determine which insertion is responsible for the altered colistin resistance phenotype. These analyses will allow us to understand the mechanism of colistin resistance in these strains and allow us to design strategies to extend the useful lifespan of colistin as a treatment antibiotic.

Dr Priscilla Johanesen

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Dr Priscilla Johanesen



Dr Geoff Dumsday



Pilot-scale facility for industrial biotechnology

Biologically-derived synthons for chemical synthesis

Dr Priscilla Johanesen and Dr Geoff Dumsday (CSIRO Materials Science and Engineering)

White biotechnology (also known as industrial biotechnology) is the application of biotechnology in industrial processes. This cross-disciplinary emerging area of science is well placed to play a key role in a sustainable future through (i) overcoming dependence on non-renewable feedstocks such as crude oil, (ii) new environmental remediation technologies and (iii) a greatly reduced environmental footprint from manufacturing. Broadly applicable across a range of market sectors, many large companies are seeking to develop products and processes that are inspired by biological systems and use renewable feedstocks such as terrestrial biomass to ensure their long term future. Some example applications of white biotechnology already proven at an industrial scale include production of 1,3-propanediol (DuPont), polylactic acid (NatureWorks LLC) and more recently isoprene (Genencor) to name but a few. Not only are these products derived from renewable feedstocks, but the processes typically require less energy input, less solvents and result in reduced levels of toxic by-products.

Our research centres on industrial biotechnology and aims to use microbial systems to specifically modify highly functional small molecules which can then be used as starting materials in a diverse array of chemical syntheses. Using a patented microbial discovery technology in combination with microbiological and molecular techniques, the student will characterise microorganisms that have the capability to use synthetically useful compounds as a sole source of carbon. Through the manipulation of growth conditions in both batch and continuous culture, the student will use a range of analytical techniques (e.g. NMR and mass spectrometry) to identify metabolic products that are potential chemical synthons. The project will provide the student with the opportunity to engage with varied scientific disciplines including industrial microbiology, molecular biology and organic chemistry and also an opportunity to work in CSIRO's recently completed state-of-art Recombinant Protein Production Facility.

Associate Professor Hans Netter

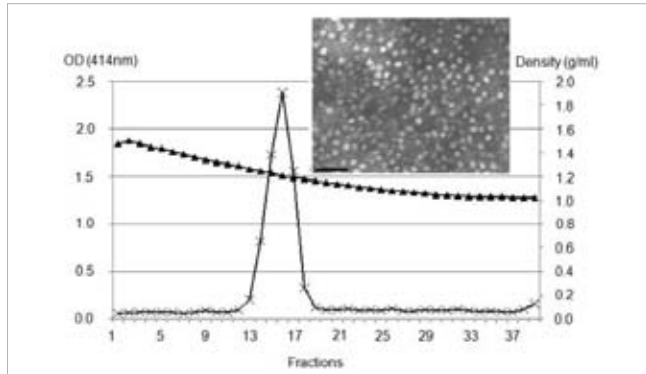
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Associate Professor Hans Netter



Dr Wan-Shoo Cheong



Purification and EM of VLPs

www.med.monash.edu.au/microbiology/research/netter.html

Modified HBV VLPs as vaccination tools against infectious diseases

Dr Wan-Shoo Cheong and Associate Professor Hans Netter

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 and T cell 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 antibody and T cell 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 antigenic determinants derived from pathogens with unmet

medical need or antibody domains for targeting purposes. Also, projects are available to determine the quality of the immune response at the level of the innate and adaptive immune system, such as uptake and processing by antigen-presenting cells. As VLPs resemble native viral structures, the outcomes have also direct implications for virus-immune system interactions.

Projects are available i) with the focus on modified VLPs and the assessment of their immunogenicity, including targeting studies of recombinant VLPs to antigen processing cells and ii) to decipher degradation pathways with relevance to innate and adaptive immunity.

Expression and characterisation of Hendravirus proteins

Dr Wan-Shoo Cheong, Professor Linfa Wang (CSIRO) and Associate Professor Hans Netter

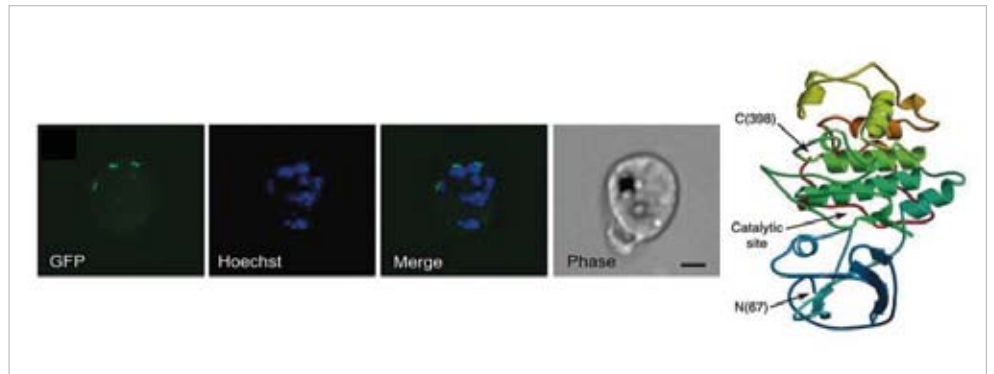
Hendravirus (HeV) is a highly pathogenic zoonotic paramyxovirus that has recently emerged in Australia. Due to its recent emergence, HeV proteins are poorly characterised, and their biochemical characteristics and molecular functions are hypothesised based on our understanding of other paramyxoviruses. In collaboration with Professor Linfa Wang, CSIRO Australian Animal Health Laboratories, projects are available to determine cellular interaction partners of HeV proteins, and to investigate their contribution to the viral life cycle. The identification of cellular factors and their role in facilitating viral replication will lead to a profound understanding of HeV pathogenesis.

Professor Christian Doerig

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Professor Christian Doerig



Malaria parasite expressing a tagged kinase

Kinomics of the human malaria parasite *Plasmodium falciparum*

Malaria remains a major public health problem in the developing world, and the emergence and spread of drug resistance of *Plasmodium falciparum*, the parasitic protist responsible for the most virulent form of the disease, make the development of novel therapeutic tools an urgent task. The research programme of our team is aimed at identifying and characterising *P. falciparum* and host protein kinases involved in the control of parasite proliferation and development. The laboratory has brought internationally recognised contributions to the field of signal transduction, cell cycle control, and kinome characterisation in malaria parasites, and has extensive expertise in protein kinase biochemistry and reverse genetics. Active collaborations have been established with pharmacology and structural biology laboratories with the purpose of developing drug discovery activities based on plasmodial protein kinase inhibition.

We have recently completed a kinome-wide reverse genetics study that identified 36 protein kinases as playing a crucial role for completion of the proliferative cycle in erythrocytes. In most cases, the molecular and cellular function of these essential enzymes, and the

physiological processes that they regulate, remain unknown. One way to gain information in this respect is to identify the components of protein complexes involving these enzymes, by implementing protein-protein interaction studies. Parasite clones have been established, in which individual kinase-encoding genes have been modified so as to add an epitope tag to the C-terminus of the kinase. The project will consist of: (i) growing transgenic parasite lines each of which expresses a different epitope-tagged kinase; (ii) verify their genotype at the relevant locus; (iii) monitor expression of the tagged enzyme using western blot analysis and immunofluorescence; (iv) identify interacting proteins through a coupled immunoprecipitation/proteomics approach, using the tag to specifically purify complexes from infected red blood cell cultures.

This will be achieved under the close supervision of a senior researcher. Honours students will have the opportunity be exposed to a wide range of approaches and techniques, including parasite culture, genotyping by PCR and Southern blot, western blot, immunofluorescence, and close interactions with the proteomics platform.

Projects based at affiliated institutions

Monash Institute of Medical Research



Associate Professor
Richard Ferrero

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The Ferrero Group

www.monashinstitute.org/centres/ciiid/gastrointestinal_infection_inflammation.html

Helicobacter pylori interactions with host cells: the role in innate immunity and inflammation

The gastrointestinal tracts of mammals are colonised by a wide variety of *Helicobacter* species, some of which have been associated with chronic inflammatory conditions of the stomach (gastritis) or intestine (colitis). One of these bacterial species, *Helicobacter pylori*, was first identified nearly 30 years ago by two Australian clinicians, Drs Marshall and Warren. These researchers received the Nobel Prize in 2005 for the discovery of a link between *H. pylori* and the development of peptic ulcer disease and stomach cancer in humans.

A hallmark of *H. pylori* infection is the chronic gastritis that precedes these severe diseases. The major research theme in our laboratory is focused on understanding how and why *H. pylori* induces this inflammation. A broader interest of the laboratory is to use *H. pylori* and other infection models to address key questions relating to the mechanisms whereby pathogens engage with the innate immune system to cause inflammation in their hosts.

New *H. pylori* factors involved in host-pathogen interactions from 'next gen' sequencing data

Associate Professor R Ferrero

Despite nearly three decades of research, we still do not have a complete understanding of the mechanisms and factors by which *H. pylori* interacts with host cells and induces inflammation. In order to define such new virulence factors, we have performed 'next gen sequencing' of a pathogenic clinical isolate, *H. pylori* 10700, and its mouse-adapted progenitor, SS1. The latter is attenuated in *in vitro* assays with human GECs suggesting some 'loss' of virulence factors in this isolate. By comparing the *H. pylori* 10700 and SS1 genomes, we identified > 1,400 single-nucleotide polymorphisms

(SNPs) in the latter, including in numerous genes involved in host-pathogen interactions. These SNP-affected alleles were then shuttled back into *H. pylori* 10700 by molecular techniques and the resulting mutants studied *in vitro* and *in vivo*. In certain cases, there was a loss of phenotype associated with the SNP. The aim of future work is to investigate the impact of other SNPs of interest on *H. pylori* virulence in *in vitro* and *in vivo* models.

Techniques: molecular biology (i.e. cloning, PCR, sequencing); genetic manipulation of *H. pylori*; culture of primary and immortalized GECs; cytokine ELISA; qRT-PCR and mouse infection studies.

The role of a new NF- κ B protein family member in epithelial cell responses to *H. pylori*

Associate Professor R Ferrero and Dr A Banershee/ Professor S Gerondakis, The Burnet Institute

The aim of this project is to determine the relative contributions of different NF- κ B proteins to the pro-inflammatory signalling cascade induced by *H. pylori* in epithelial cells. From preliminary studies, we have observed that in mouse gastric epithelial cells, *H. pylori* bacteria induce the activation of the NF- κ B protein, c-rel, whereas in human cells the bacteria preferentially induce the activation and nuclear translocation of the p65 protein. c-rel is a non-canonical NF- κ B protein, whose role in inflammation is still not clearly defined. Thus, we wish to: 1) determine the relative roles of p65 and c-rel in the regulation of pro-inflammatory responses to *H. pylori* in mouse and human gastric epithelial cells and 2) elucidate the specific bacterial factors important for c-rel activation.

Techniques: the culture of primary and immortalised cell lines; cytokine ELISA and qRT-PCR; high content imaging; immunofluorescence and mouse infection studies.

H. pylori activation of innate immune responses via the 'inflammasome' and the role of cholesterol in these responses

Associate Professor R Ferrero, Dr M Liaskos and Dr B Croker, The Walter and Eliza Hall Institute

The NOD-like receptors (NLRs) consist of a family of cytoplasmic signalling molecules of the innate immune system that recognise pathogens and host-derived stimuli from damaged tissues. They share structural features but respond to different microbe stimuli and host molecules. A subfamily of NLRs, known as NALPs, are involved in the formation of various types of signalling platforms ("inflammasomes") whose principle functions are inflammatory cell death and pro-inflammatory cytokine processing and responses involving IL-1 β and IL-18. Each member of the NALP family responds to either pathogen- or host-derived stimuli e.g. bacterial toxins, flagella, cholesterol or uric acid crystals. Although *H. pylori* is known to induce IL-1 β and IL-18 responses in host cells, no studies to date have explored the role of inflammasome proteins in these responses. Preliminary data from our laboratory suggest that cholesterol, which is a stimulus for inflammasome activation, accumulates within the *H. pylori* cell wall and may thus contribute to host inflammatory responses against the bacterium. We therefore wish to define which NALP proteins are activated by *H. pylori* in epithelial cells or macrophages, as well as the potential role of bacterial-associated cholesterol in this activation. For this, we will use mice deficient in specific NALP or adaptor molecules.

Techniques: the culture of primary epithelial cells and macrophages; cytokine ELISA; Western blotting; qRT-PCR; mouse infection studies and immunohistochemistry on mouse tissues.

Characterisation of a new NLR and its role in *H. pylori* inflammation

Associate Professor R Ferrero and Dr J Ferrand, Monash Institute of Medical Research

Recently, a new member of the NLR family, NLRC5, has been described. The limited findings to date regarding the role of NLRC5 in innate immune responses to microbial pathogens have thus far been contradictory. Indeed, some studies described NLRC5 as being a negative regulator of NF- κ B-dependent responses, whereas other studies attributed a role for NLRC5 in pro-inflammatory responses. From preliminary studies, we know that NLRC5 is expressed in GEC lines as well as in biopsies from individuals with *H. pylori*-associated gastritis. It also appears that *H. pylori* can up-regulate NLRC5 expression in these cells. The aim of the project is to investigate the role of NLRC5 in host cell responses to microbial infection. For this, we will use conditional *nlr5* knock-out mice, which have been generated for the first time in Associate Professor Ferrero's laboratory at MIMR.

Techniques: the culture of primary GECs and macrophages; cell transfection; cytokine ELISA; qRT-PCR and mouse infection studies.

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: 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 Robert Moore

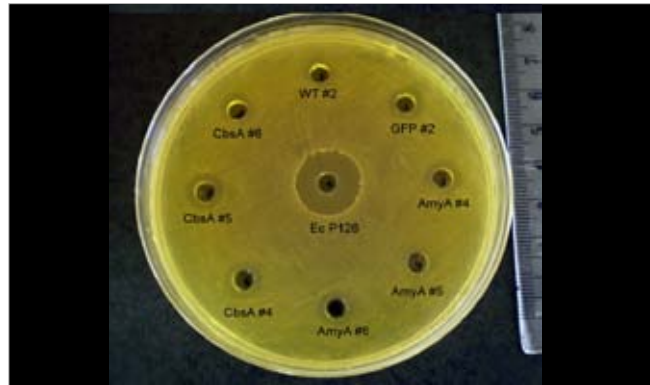
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Dr Robert Moore

Bioengineering *Lactobacillus* strains to deliver recombinant therapeutic proteins

We have screened a large collection of *Lactobacillus* isolates to identify a few special strains that are able to reliably colonise and persist in the gastrointestinal tract of chickens. Such persistent strains can be used to deliver therapeutic proteins to the gut of chickens. This project will investigate ways of bioengineering these strains to produce recombinant proteins such as vaccine antigens, cytokines and antimicrobial proteins. The activities of gene expression sequences (eg. promoters and secretion signals) in *Lactobacillus* are often quite strain specific. We have determined the whole genome sequence of our



Bioassay for antimicrobial activity

most favoured *Lactobacillus* strain and this project will mine the genome sequence for useful promoters, secretion signals and terminator sequences for incorporation into gene expression cassettes. Selected therapeutic proteins will be expressed and the constructs will be tested for their biological impact when delivered to chickens. The student will have the opportunity to learn bioinformatic methods of screening whole genome information for useful sequences. They will learn basic microbiological techniques for both aerobic and anaerobic bacteria, as well as molecular techniques such as how to clone and express genes, evaluate protein production and perform biological assays for protein function.

Dr Anthony Keyburn
and Dr Robert Moore

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Drs Anthony Keyburn and Robert Moore

The role of NetB toxin in disease causing isolates of *Clostridium perfringens*

Necrotic enteritis is a significant disease in chickens that costs the industry \$2 billion dollars each year. The causative agent is *Clostridium perfringens*, a Gram-positive spore-forming anaerobe found as normal residents in the gastrointestinal tract of humans and animals. A new toxin discovered in our laboratory, NetB, is essential for virulence. However, the virulence of NetB positive strains varies considerably. This project will investigate the role



Necrotic enteritis disease in chicken gut

of NetB in strains that cause high levels of disease compared to strains that cause moderate to low levels of disease. Specific gene knock-out mutations will be constructed and biologically tested. The project will involve a considerable amount of molecular techniques including PCR, cloning and sequencing as well as microbiology techniques including mutagenesis and isolation and culturing of anaerobic pathogens. Animal handling and disease modelling will also be a part of the project in a unique world class facility.



Associate Professor Heidi Drummer

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Associate Professor Heidi Drummer

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

Hepatitis C Virus infects 3 per cent 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. Within E2 is a discrete receptor binding domain encompassing the receptor binding site and major neutralization epitopes. Glycoprotein E2 comprises three variable regions that alternate with conserved regions. 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 sequence evolution within the variable regions during natural HCV infection impacts on the ability of antibodies to neutralize virus. This project will require work in both PC2 and PC3 laboratories following training.

Expression of recombinant hepatitis C virus E1E2 heterodimers

The HCV glycoproteins, E1 and E2, mediate virus attachment and membrane fusion and are present as both disulphide linked and non disulphide linked heterodimers. There is no structural information available for either E1, E2 or the E1E2. The C-termini of E1 and E2 contain transmembrane domains (TMDs) that anchor the glycoproteins to the surface of the virus. The TMDs also act to retain the glycoproteins within the endoplasmic reticulum and are essential for heterodimerization of E1 and E2. Whilst removal of the TMDs result in high level expression of soluble ectodomains suitable for structure determination studies, the glycoproteins can no longer form heterodimers. We have replaced the TMDs of E1 and E2 with soluble leucine zipper domains that heterodimerize. These recombinant soluble E1E2 heterodimers retain the ability to bind the cellular receptor CD81 and are recognised by conformation dependent antibodies. This project will construct new E1E2 heterodimers from different HCV genotypes, further characterise the recombinant E1E2 heterodimers and explore methods to produce purified forms suitable for structural studies. This project will require work in a PC2 facility.

Associate Professor
Gilda Tachedjian,
Dr Con Sonza and
Dr Jenny Anderson

One vacancy

Retroviral Biology and Antivirals Laboratory
(RBA) (Tachedjian Lab)

Centre for Virology

Macfarlane Burnet Institute for Medical Research
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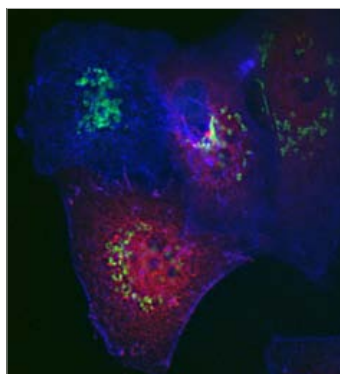
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Associate Professor Gilda Tachedjian



Autophagy protein (red) in cells in relation to Golgi (green) and F Actin (blue)

www.burnet.edu.au/home/cvirology/retroviralbiologyandantivirals

The Retroviral Biology and Antivirals Laboratory undertakes fundamental research to understand the biology of retroviruses (eg. HIV) including the role of host cell factors and viral proteins in retroviral replication. We also study the role of HIV mutations selected during antiretroviral therapy in drug resistance and undertake translational studies to identify, determine the mechanism of action, and develop novel agents for the treatment and prevention of HIV and other sexually transmitted infections.

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

Characterisation of retroviral sequences in a native Australian mammal

**Associate Professor Gilda Tachedjian,
Mary Tachedjian (CSIRO Geelong) and
Professor Linfa Wang (CSIRO Geelong)**

Retroviruses such as gammaretroviruses have simple genomes, in contrast to HIV, and are known to infect a wide variety of species including mice, cats, koalas and non-human primates and cause leukemias, lymphomas, neurological diseases and immunodeficiencies in these species. Retroviruses are found in the genome of mammals and can be transmitted vertically through the germ line (ie. endogenous) or transmitted horizontally (ie. exogenous). Endogenous retroviral sequences are present as a critical part of eukaryotic genomes. While the majority of the sequences are defective, a few are capable of producing infectious virus spontaneously upon long-term cell culture or by treatment with chemicals. Analysis of the transcriptome of a native Australian mammal has revealed the presence of retroviral transcripts that, at

the amino acid level, demonstrate homology to extant (currently existing) retroviruses found in mice, cats and koalas. However, whether this species harbours an infectious retrovirus is unknown. The aims of this study are to characterize the retroviral sequences found in the transcriptome of the Australian mammal and to determine whether cell lines derived from the Australian mammal release retroviral particles. Viral RNA will be isolated from virions, the nucleotide sequence and genomic organization determined and an infectious molecular clone constructed.

Mechanism of Action of Dendrimer Microbicides against HIV

Dr C Sonza and Associate Professor G Tachedjian

Female initiated strategies are urgently required to prevent women acquiring HIV through sex. One strategy that has gained considerable attention 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). Proof of concept that a gel based topical microbicide prevents HIV acquisition in women has recently been described (Karim et al 2010 Jul 19 Epub ahead of print). We have been involved in the preclinical development of a dendrimer microbicide, SPL7013, which has broad-spectrum anti-HIV and anti-HSV activity. Dendrimers are chemically defined nanoparticles comprising a central core, branches and are decorated with surface groups that impart their biological activities. Our studies show that SPL7013 inhibits HIV that utilise the CXCR4 and CCR5 chemokine receptors in cell culture assays at similar concentrations (Tyssen et al PLoS ONE accepted July 2010). Intriguingly, we have also shown that

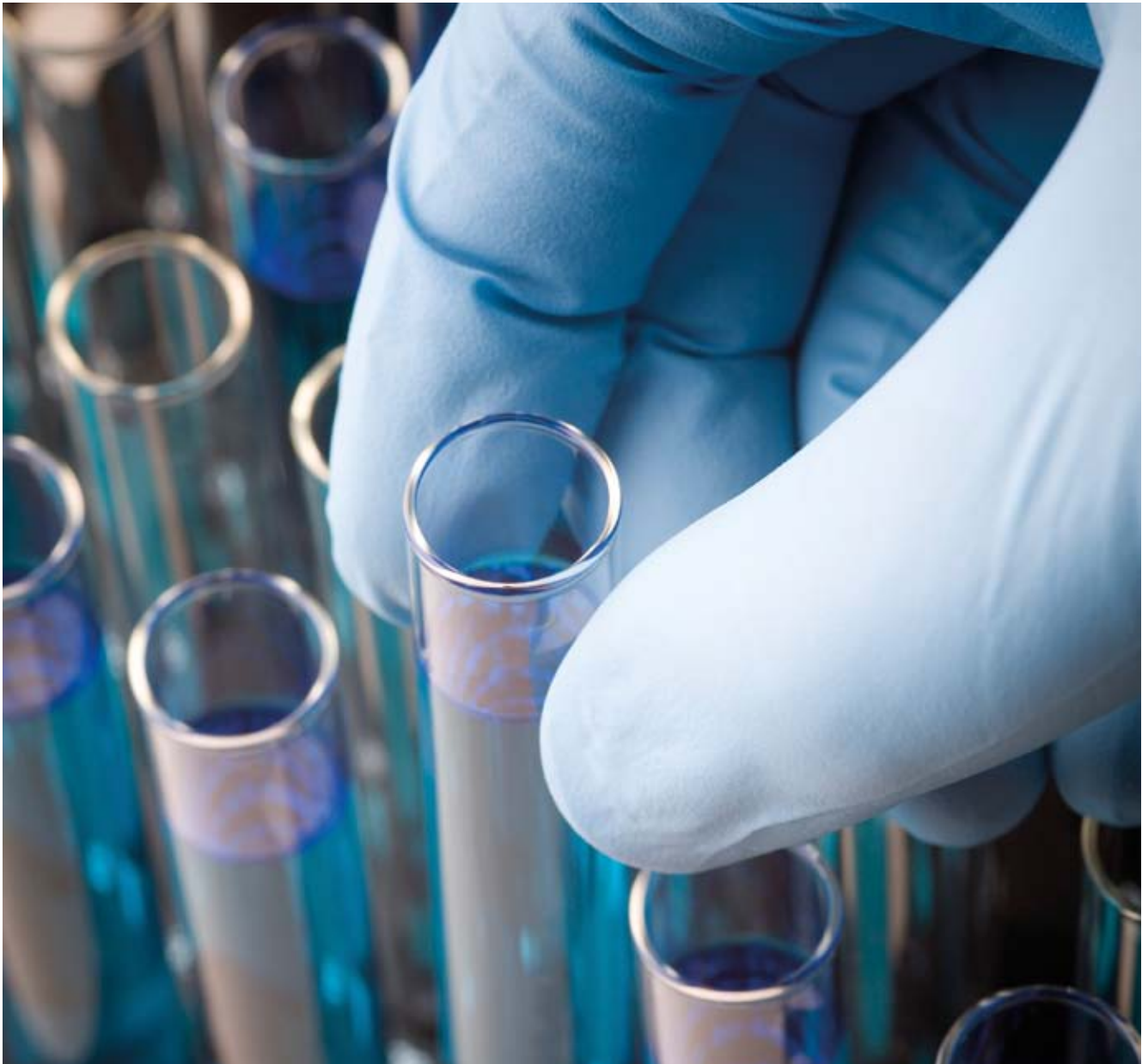
SPL7013 has direct virucidal activity against CXCR4 but not CCR5 strains indicating that the SPL7013 mechanism of action for these HIV strains is distinct (Telwatte et al 2011 Antiviral Res 90:195). The aim of this study is to determine why SPL7013 is virucidal against CXCR4 using but not CCR5 using HIV strains. This study will lead to a greater understanding of how dendrimer nanoparticles block HIV infection.

Host Cell Proteins Required for HIV Replication

Dr C Sonza, Dr J Anderson and Associate Professor 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 a host cell protein involved in autophagy interacts with the HIV reverse transcriptase (RT). In addition, the abovementioned siRNA knockdown study showed that this and other autophagy proteins are required for HIV replication however, their precise role in the virus life-cycle remains to be elucidated. In this project siRNA knockdown studies will be performed to determine the role of these autophagy proteins in HIV replication. These studies are important to understand the basic replication strategy of HIV and to identify potential drug targets.

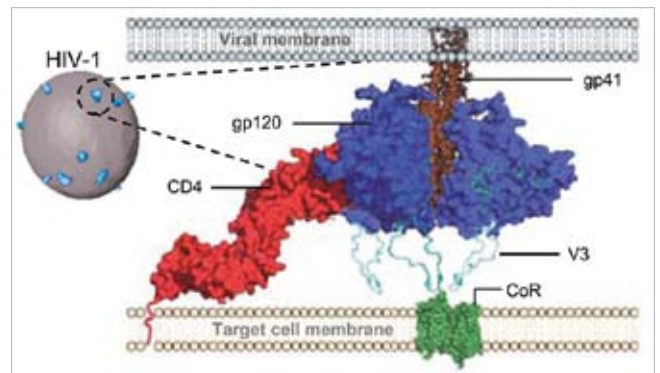


Associate Professor Paul Gorry



Associate Professor Paul Gorry

One vacancy
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HIV Env glycoproteins interacting with receptors

The Gorry laboratory studies the mechanisms of HIV-1 entry into cells, with the goal of understanding adaptive changes in the HIV-1 gp120 envelope glycoproteins (Env) that are important for HIV-1 pathogenesis. Specific research areas include structure-function studies of the interactions between gp120 and cellular entry receptors (CD4 and the coreceptors CCR5 or CXCR4, see Figure), the pathogenesis of HIV-1 subtype C, determinants of HIV-1 tropism for macrophages, the mechanisms of resistance to HIV-1 entry inhibitors, and mechanisms of HIV-1 neurotropism.

Mechanisms of HIV-1 resistance to CCR5 antagonists

Associate Professor Paul Gorry and Associate Professor Melissa Churchill

HIV-1 entry into cells is initiated by the interaction between the viral gp120 Env glycoproteins and CD4 expressed on the target cell surface. CD4 binding triggers a conformational change in gp120 that exposes the binding site for a coreceptor, either CCR5 or CXCR4. Maraviroc (MVC) belongs to a new class of anti-HIV drugs which inhibit the interaction between CD4-bound gp120 and

CCR5. Maraviroc and other CCR5 antagonists achieve this by binding to the transmembrane helices of CCR5 which alters the conformation of the CCR5 extracellular loops. Thus, they inhibit gp120 binding to CCR5 via an allosteric mechanism by stabilising CCR5 in a conformation that is no longer recognised by gp120. As with all anti-HIV-1 drugs, resistance to MVC can develop and lead to virological failure. Our recent studies suggest that when HIV-1 escapes inhibition by MVC, the virus becomes less fit and is less able to establish infection in macrophages, which are an important tissue reservoir of HIV-1. This project will characterise the mechanism of HIV-1 resistance to MVC in patients who commenced and ultimately failed MVC therapy, with an emphasis on determining structural alterations in the Env glycoproteins associated with resistance and attenuated macrophage infectivity. In addition to elucidating clinically-important resistance mechanisms, these studies have the potential to identify novel ways to reduce the macrophage burden of HIV-1, thus contributing to the current global strategy that aims to purge HIV-1 from persistent reservoirs and effectively cure HIV-1 infection.

Monash Microbiology Honours 2012

Name:

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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 25, 2011



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