FACULTY OF MEDICINE, NURSING & HEALTH SCIENCES

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

SCHOOL OF BIOMEDICAL SCIENCE

CLAYTON CAMPUS

Information on Research Programs
for
2013 Honours and Postgraduate Students
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1. Cell Signaling & Cancer

Proteases, serpins and cytolysins in cell development and death

Professor Phil Bird

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A. Granzymes and perforin

Granzymes are proteases produced by cytotoxic lymphocytes. Cytotoxic lymphocytes (Fig 1) destroy virus-infected or cancer cells by releasing granzymes, which enter the cytoplasm via the pore-forming cytolysin perforin to trigger apoptosis. Granzyme B activates caspases, and is one of the most lethal proteases known. How perforin helps it enter the target cell is unclear.

B. Regulation of proteases by serpins

Serpins trap and inactivate proteases. Serpin deficiency results in blood clots, immune dysfunction, lung disorders, cancer and dementia. Some serpins found inside cells protect protease-producing cells against their own proteases, to prevent unwarranted or untimely death. For example, we have shown that the serpin, PI-9, prevents suicide of cytotoxic lymphocytes caused by exposure to their own granzyme B (Fig 2).

C. Perforin-like molecules in neural function and cancer

Bone Morphogenetic Protein – Retinoid Acid Inducible Neural Specific Proteins (BRINPs) are ancient, highly conserved proteins related to perforin but their molecular roles are entirely unknown. They are found mainly in the vertebrate nervous system (Fig 3), and BRINP1 is tumour suppressor gene commonly lost in astrocytomas and bladder cancers.

Examples of project areas for Honours students

We use advanced techniques in molecular cell biology to uncover the roles of the above proteins in health and disease. These include recombinant protein production and analysis, gene manipulation, RNA interference, proteomics, bioinformatics, cell culture and confocal imaging, protein crystallography, and the analysis of model organisms such as "knockout" mice and zebrafish. Projects are available in the following areas:

1. Granzymes. Granzymes function beyond cytotoxicity, in cytokine signaling and cell migration. Granzyme inhibitors may therefore be useful as anti-inflammatory drugs. We produce recombinant granzymes in yeast or E.coli, for analysis using molecular approaches and protein crystallography. Substrates are identified using proteomics, and potential inhibitors are identified by phage display, model peptides and bioinformatics. Uptake into cells is monitored by confocal microscopy and FACS. Granzyme roles in vivo are studied using knockout mice.

2. Serpins, lysosomes and cell death. Lysosomes act as "stress sensors", and release of lysosomal proteases into the cytoplasm causes cell death. In cytotoxic lymphocytes, release of granzyme B from granules (specialized lysosomes) causes death once it exceeds PI-9 levels (Fig 2), and hence limits their life-span. We use cells from knockout mice, and approaches including live cell imaging, to understand how stress releases granzyme B from granules, how PI-9 controls this, and the consequences for the immune system of the animal.

3. Perforin and BRINPs. With Prof J. Whisstock and Prof J. Trapani (Peter MacCallum Cancer Institute) we are studying perforin function, in particular how it helps granzymes enter a target cell. We have characterized BRINP genes from mouse and zebrafish, and have generated knockout mice. We are using these mice and zebrafish (Fig 3) to study the expression, localisation and role of BRINPs in developing and mature neural tissue, and the effect of disrupting their function. We are also searching for BRINP binding partners. Zebrafish projects are co-supervised by Dr. H. Verkade (School of Biological Sciences).
**Endocrine Signaling, Lung Development and Cancer**  
A/Prof. Timothy J. Cole; Email: tim.cole@monash.edu

The steroid hormones cortisol (a glucocorticoid) and aldosterone (a mineralocorticoid) are secreted by the adrenal gland and are important in the systemic physiology and homeostasis of humans and other mammals. Cortisol has many supportive and homeostatic roles in a wide range of tissues both during embryogenesis, particularly the developing lung, and in the adult. In abnormal states, such as during high stress, injury or infection increased circulating levels of cortisol act to blunt harmful systemic responses such as the activated immune system. Glucocorticoids exert their effects by binding to the intracellular glucocorticoid and mineralocorticoid receptors, GR and MR respectively. Both are members of the nuclear receptor superfamily of ligand-dependent nuclear transcriptional regulators. Research projects below will utilize a range of molecular, biochemical and genetic techniques in both cell-based and animal systems to investigate these cell signaling pathways and their specific roles development and cancer.

**Honours Projects:**

1. **Selective Glucocorticoid Receptor Modulators (SGRMs) for treatment of preterm birth:**
   Novel steroid-like compounds are being developed that have potent selective effects via the GR in specific tissues such as the liver, brain and respiratory system. These compounds bind to the GR and modulate interactions in the nucleus of cells to allow regulation of particular sets of down-stream target genes. This project will test a range of new SGRM compounds in lung cell lines, lung explants cultures and *in vivo* with mice. Selected compounds may provide more specific treatments for very preterm birth, and in patients with diabetes or metabolic syndrome.

2. **Glucocorticoid and cAMP signaling pathways in embryonic lung development and respiratory diseases:**
   Lung dysfunction in adults and at birth is a major cause of morbidity and mortality. Systemic hormones such as retinoic acid, glucocorticoids and cAMP signaling play an important role in embryonic lung development. We have a number of mouse gene-knockouts that interrupt the cell signaling of these hormones. These include mouse knockout lines of GR, CREB, HSD1 and RARα (1, 2). These mice develop perinatal lung dysfunction and will be used to investigate the specific molecular and cellular role each hormone/receptor pathway plays during fetal respiratory development. We are utilizing the Cre-recombinase/loxP gene recombination system in mice to produce cell-type-specific gene knockouts in the developing lung. This will identify specific endocrine actions of these pathways in mesenchymal, epithelial and endothelial cell compartments.

3. **Steroids/HSD enzymes and human cancer:**
   Steroid signaling pathways are often down-regulated in cancer cells either by reducing steroid receptor levels or by increasing expression of other modifying proteins. A novel human steroid dehydrogenase will be analyzed in human cancer cells for levels of expression and substrate specificity in comparison to normal cells.

4. **The function of a novel RAB protein in lung development and disease:** (Supervisors: A/Prof. Tim Cole and Prof. Moira O’Bryan-Anatomy) Through the use of a random mutagenesis we have identified a novel regulator of cilia function in the testis and lung. Homozygous mutant mice display male sterility and lung disease consistent with aspects of human primary ciliary dyskinesia. In addition however, older mice accumulate protein within alveolar spaces suggestive of perturbed surfactant production. An honours project exists to define the cellular and biochemical basis of the lung disease. Techniques to be used include: immunohistochemistry, electron microscopy, PCR, Western blotting, immunoprecipitation and the use of an animal model.  
"Collaborators: Prof. Stuart Hooper, Dr. Tim Moss, Monash Medical Center and Prof. Richard Harding, Anatomy & Dev. Biol., Prof. Peter Davis, Royal Women’s Hospital, Parkville."
Cell Biology and Cancer Research

The ability of migrating cells to find their genetically programmed positions is essential for normal development and its de-regulation an important feature of metastatic cancers. Members of the Eph receptor tyrosine kinases together with cell surface bound ephrin ligands as a ‘global positioning system’ of the body that directs cells to their correct positions. Not surprisingly, the unscheduled function of Ephs is implicated in growth and spreading of cancers. In our laboratory we aim to understand the cellular and molecular concepts of this cell positioning system, and to use our insights to develop new approaches to combat cancer growth. Our most advanced therapeutic, the anti-EphA3 antibody KB004, targets leukemias, as well as the tumor vasculature in solid cancers. The KB004 antibody recently entered clinical trials in cancer patients. It also remains an important tool for our continuing studies. We pursue research projects in cell biology and cancer research, and use advanced molecular, biochemical and cell biological approaches and imaging strategies to study oncogenic functions of Eph receptors in cultured cells and in live animals.

Cell Biology

Molecular and cellular mechanisms underlying EphA3-guided blood-vessel assembly.

Coordinated cell navigation is critical for normal tissue assembly and is deregulated in cancer. Eph RTKs and cell surface ephrin ligands are essential positioning cues that act by modulating either cell-cell adhesion or repulsion: Simplistically, Eph kinase activation triggers shedding of ligated ephrins, prevents cell-cell adhesion and favours segregation, while lack of kinase activity causes cell-cell adhesion. We are using in-vitro and in-vivo models of neo-angiogenesis to understand how the coordinated response of newly recruited endothelial and perivascular progenitor cells to these dichotomous Eph functions enables the formation of new blood vessels. In collaboration with P. Bastiaens we are developing biosensors that allow visualising the activity of Eph/ephrin signalling nodes in these models by functional imaging approaches.

The molecular mechanics of Eph-induced cell-cell segregation.

The default response to Eph kinase activation by cell surface ephrins is cell-cell segregation, and Eph kinase inactivating mutations identified in various metastatic cancers cause de-regulated adhesion and identified EphA3 as candidate cancer gene. Cell-cell segregation relies on controlled shedding of ephrins and endocytosis of Eph/ephrin complexes, followed by coordinated very rapid cytoskeletal contraction of both cells. Although a large number of signalling proteins implicated in Eph signalling are known, the mechanisms that link ephrin shedding and Eph activation to the synchronised cytoskeletal re-organisation of retracting neighbouring cells has remained obscure. We are using cell biological and imaging approaches to monitor trafficking of Eph and ephrin signalling clusters in intact cells, and exploit molecular biology and proteomics strategies for identification and functional characterisation of the involved signalling proteins.

Converging Eph and EGF-receptor signalling pathways.

Eph and EGFR RTKs have key roles in normal and oncogenic development, amongst other functions control cell positioning, proliferation, survival and differentiation. Both receptors are commonly co-expressed in a range of tumours and merging evidence indicates crosstalk between the two RTKs and convergence of their endocytic signalling pathways. Importantly, both RTKs are regulated by the same ADAM transmembrane metalloproteases, which facilitate endocytosis and signalling of Eph/ephrin complexes during cell-cell repulsion, and processing of EGFR ligand precursors as prerequisite of EGFR activation. Ongoing studies examine the fate of Eph and EGFR signalling complexes after ADAM-mediated ligand cleavage and subsequent receptor-mediated endocytosis in the context of tumour cell positioning and tumour cell proliferation.
Cancer Research

Targeting the EphA3 patterning function in the tumour microenvironment.

A/Prof. M. Lackmann and Dr. Mary Vail (Mary.Vail@monash.edu).
Collaborators: Prof. A. Scott, Ludwig Institute Melbourne; KaloBios Pharmaceuticals, San Francisco.

Tumour growth and metastasis rely on Eph-mediated communication between cancer and host mesenchymal cells. EphA3, classified as tumour suppressor and candidate cancer gene, is expressed on mesenchymal cells within the tumour margin, whereby our therapeutic α-EphA3 antibody KB004 inhibits tumour growth by disrupting tumour microvasculature and stroma. By exploiting a range of tumour models and transgenic mice to allow conditional EphA3 silencing, our studies aim to understand the role of EphA3 during recruitment of the mesenchymal stromal cells from the bone marrow and during assembly of the tumour stroma and vasculature. They are critical to define the identity of EphA3+ bone marrow-derived cells as targets of the therapeutic anti-EphA3 mAb and thus will help to develop protocols for the planned clinical trial in solid tumour patients.

Therapeutic mechanisms of the anti-EphA3 mAb in haematopoietic cancers.

A/Prof. M. Lackmann & Dr. Rae Farnsworth (Rae.Farnsworth@monash.edu).
Collaborators: Prof. R. Lock, Lowy Cancer Research Centre, Sydney, KaloBios Pharmaceuticals San Francisco.

EphA3 is prominently overexpressed on a large proportion of haematopoietic tumours, including AML, CML, CMML, PV, IM, but is absent from normal blood cells or adult human tissues. The therapeutic antibody against EphA3, KB004, is currently in Phase1 clinical trials in these cancers. This agonistic antibody, a recombinant, humanized version of our anti-EphA3 mAb, has pro-apoptotic and enhanced antibody-dependent cellular cytotoxicity (ADCC) activity against various hematologic cancers and shows selective inhibition of colony formation from long-term culture-initiating cells (LTC-ICs) in primary leukemia samples, suggesting its specific targeting of leukemic stem cells. We will study primary leukemic cells in xenografted mice, tissue and bone marrow samples from cancer patients, together with leukemic cell lines and mouse models to study the role of EphA3 during disease progression and the tumour cell killing mechanism of the KB004 antibody.

Development of function blocking antibodies against ADAM10 as anti-cancer therapeutics

A/Prof. M. Lackmann & Dr. Peter Janes.
Collaborators: Prof. A. Scott, Ludwig Institute for Cancer Research, Prof. D. Nikolov, Memorial Sloan Kettering Cancer Centre, New York.

The functions of key oncogenic cell surface receptors and their ligands, including Notch (and ligands), Eph, EGF and cytokine receptors depend on shedding by the ADAM transmembrane metalloproteases. Their deregulated expression and activity are one cause for the uncontrolled oncogenic signalling in hematopoietic and solid tumours. However, due to off-target effects, the development of therapeutics blocking ADAM10 activity was unsuccessful so far.

We developed an alternative inhibitor strategy, based on the ADAM10 substrate recognition module outside the catalytic domain, which provides a novel target for antibody-based inhibition of ADAM activity. One of our monoclonal antibodies, mAb 8C7, specifically binds the ADAM10 substrate recognition pocket, blocks ADAM-mediated RTK signalling and inhibits the growth of human tumour xenografts in a mouse model. We pursue the preclinical development of the 8C7 mAb as inhibitor of ADAM10-mediated cancer progression and potential anti-cancer therapeutic.
Cells respond to changes in their microenvironment by activation of complex signalling cascades. The phosphoinositide 3-kinase (PI3K) signalling pathway is involved in a number of cellular processes such as cell growth, survival, migration and differentiation. PI3K is a proto-oncogene and deregulation of the PI3K pathway occurs in human diseases including cancer, diabetes and neurodegenerative diseases as well as developmental disorders.

Our laboratory focuses on a family of enzymes known as inositol polyphosphate phosphatases which are critical regulators of the PI3K pathway and regulate many cellular functions. Mutation or altered expression of inositol polyphosphate phosphatases has been detected in human diseases such as Joubert and Lowe syndromes, breast cancer, insulin resistance, leukaemia and degenerative neuropathies.

We use a wide range of cutting-edge molecular, cell biology and protein chemistry techniques to delineate the role of inositol polyphosphate phosphatases in human disease. Some of the techniques used include: cell culture, fluorescence, confocal and electron microscopy, immunohistochemistry (IHC) utilising samples from human patients as well as mouse tissues, RT-PCR and antibody generation. We have also developed knockout and transgenic mice as models to investigate the role the inositol polyphosphate phosphatases play in development and disease as well as cancer progression and metastasis.

Honours projects available for 2013 include the following:

**Characterising the role of a PI3K regulatory enzyme in medulloblastoma and other cancers**
Contact: Christina.Mitchell@monash.edu and Jennifer.Dyson@monash.edu

Medulloblastoma is the most common malignant brain tumour in children and has devastating effects on patient wellbeing and survival. Medulloblastoma is highly aggressive and there is a need for new therapies and therapeutic targets. The Hedgehog (Hh) signalling pathway is hyperactivated in ~60% of medulloblastomas and PI3K signalling is a major driving force in cancer progression, including brain. We have identified a PI3K-regulating protein that also regulates Hh signaling. We have exciting evidence that deletion of our gene in a Hedgehog-driven mouse model of medulloblastoma increases the latency of the disease and provides a survival advantage, suggesting our gene of interest may be a novel therapeutic target for medulloblastoma and other Hedgehog-driven cancers.

This research project will include examining the role of our gene in our mouse medulloblastoma model and determining the expression of our gene in human medulloblastoma biopsies. In addition, this project will include screening for altered expression of our gene using cancer arrays of other Hedgehog- and/or PI3K-dependent cancers and examining the role of our gene in these cancers. Applicable techniques include cell culture including protein overexpression and siRNA-mediated depletion, mouse cancer models, IHC and microscopy. Overall this project aims to identify a new therapeutic target for Hedgehog- and/or PI3K-driven cancers.

**Role of a PI3K regulatory enzyme in embryonic development, angiogenesis and cancer**
Angiogenesis is the formation of new blood vessels from a pre-existing primitive vasculature. Precise regulation of angiogenesis is critical during embryonic development and also in the adult body during reproduction and healing. However, dysregulation of angiogenesis contributes to serious human disease, including cancer, cardiovascular disease, and macular degeneration. Drugs that either inhibit or activate angiogenesis are currently being assessed in human clinical trials for therapeutic suitability in the treatment of human disease. Indeed, anti-angiogenic therapies have been approved for use in the treatment of several types of human cancer. Regulation of the PI3K signalling pathway is vital for effective angiogenesis.

We have identified a PI3K-regulatory enzyme that is essential for angiogenesis. Deletion of this gene in mice results in embryonic death; and altered gene expression is reported in various human cancers. This project will investigate the role this gene plays in angiogenesis during development and cancer using tissue-specific gene knockout mouse models, siRNA-mediated gene knockdown in endothelial cell models and human tissue samples. We propose this research will contribute to the identification of novel therapeutic targets to treat human disease resulting from defective angiogenesis.

Role of a PI3K regulatory protein in breast cancer
Contact: Christina.Mitchell@monash.edu and Lisa.Ooms@monash.edu

Breast cancer affects 1 in 9 women and an understanding of the molecular mechanisms underlying this disease is critical to improve patient outcome. The PI3K pathway is deregulated in >70% of human breast cancers and several PI3K pathway regulatory proteins have been implicated as tumour suppressor genes in this disease. Recent clinical trials using PI3K inhibitors have indicated the potential of these drugs for targeted anti-cancer therapy, therefore identifying cancers with amplified PI3K signalling is of critical importance for optimising treatment strategies.

We have shown that altering expression of PI3K pathway regulatory proteins results in increased breast cancer cell proliferation and survival in cell culture. In addition, knockout of one of these genes promotes breast cancer initiation in a mouse model of the disease. This project will evaluate the role PI3K pathway regulatory proteins play in breast cancer by analysis of protein expression and localisation in human breast cancer samples. This project will also utilise animal models of breast cancer to determine the role these PI3K regulatory proteins play in breast cancer development and metastasis in vivo.
SKELETAL MUSCLE REGENERATION AND DISEASE

Professor Christina Mitchell  christina.mitchell@monash.edu
Dr Meagan McGrath        meagan.mcgrath@monash.edu

Skeletal (striated) muscle comprises a large component of the human body and is essential for life. It facilitates voluntary movement and involuntary movements including breathing. Skeletal muscle shows an enormous plasticity and can adapt to stimuli including exercise and increased load, resulting in muscle growth (hypertrophy). Conversely, muscle wasting (atrophy) results in reduced muscle mass and occurs in diseases including cancer, AIDS, diabetes, and also with ageing. The importance of muscle to health is typified by the large number of inherited diseases, dystrophies and myopathies, resulting in progressive muscle wasting. Affected patients can become wheelchair-dependent and these diseases can be fatal. Skeletal muscle is also capable of remarkable repair following injury and is facilitated by a resident population of muscle stem cells, called satellite cells. Targeting factors which promote muscle growth (hypertrophy), satellite cell activation and/or muscle regeneration are attractive therapeutic targets not only for the treatment of acute injuries (trauma or sporting injuries) but also for conditions of chronic muscle wasting or damage as occurs in dystrophies.

Honours Projects:

1. Identification of new treatments for Duchenne muscular dystrophy: Duchenne muscular dystrophy (DMD) is the most common inherited muscle disease affecting children and death results generally in the second decade of life. There is no cure or effective treatment. DMD is caused by mutations in the dystrophin gene resulting in the absence of dystrophin protein, and subsequently fragile muscle fibres which undergo progressive damage. Utrophin is a protein which can compensate for dystrophin therefore, targeting utrophin expression is an attractive potential treatment for DMD. We have used the mdx mouse model of DMD and identified a new utrophin-regulatory pathway. Genetic manipulation of mdx mice to target this pathway, significantly reduced muscle damage and improved muscle function. This honours project will further investigate the clinical potential of this promising discovery, by identifying drugs and compounds which target our novel utrophin-regulatory pathway. These drugs will be trialled in the pre-clinical mdx model, testing their ability to reduce muscle damage. The efficacy of these drugs to promote utrophin expression will also be evaluated using muscle cells isolated directly from DMD patients.

2. Identification of novel activators of skeletal muscle regeneration: FHL1 is a newly identified protein which we have shown promotes muscle growth. We have previously generated skeletal muscle-specific FHL1 transgenic mice, which exhibit increased muscle mass (hypertrophy) associated with increased muscle strength. Interestingly we also observed that FHL1-trangenic mice had increased numbers of muscle stem cells. This honours project will determine if FHL1 is a novel protein for enhancing muscle repair. Muscle injury will be experimentally induced in FHL1-transgenic mice and the activation of muscle stem cells and efficiency of muscle repair will be examined. Additionally, the pathways by which FHL1 promotes enhanced muscle repair will be investigated.

Techniques: Transgenic and knockout mouse models, surgery, histology, immunohistochemistry, light and confocal microscopy, tissue culture, cell-transfection, siRNA, luciferase assays, ELISA, qRT-PCR, SDS-PAGE and Western blot.
Cancer Biology and Metastasis Laboratory
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Cancer currently accounts for over 30% of male deaths and 26% of female deaths in Australia. The progression of a cancer from one that is physically restricted in its growth to one that invades, spreads and proliferates at distant sites is the major cause of death in cancer patients. This process, termed metastasis, is highly complex and multi-step in nature and involves altered migration, adhesion, invasion, survival and growth of the cancer cell.

The chief aim of the laboratory is to identify novel molecules involved in metastasis and their functional significance in a hope to identify novel therapeutic targets. In addition, we are also currently testing the efficacy of new experimental compounds and drugs upon cancer growth and metastasis with a particular focus upon bone metastasis. To achieve these research goals we employ a wide range in molecular and cellular techniques, as well as a number of in vivo (xenograft metastasis mouse models) models.

**Project Areas**

**Novel Molecular Modulators of Breast Cancer Growth and Metastasis.** To model metastasis, our laboratory has utilised human breast cancer cell lines, intra-cardially injected into mice as a system to study bone and visceral organ metastasis. Using this methodology we have isolated sublines and subclones of the human breast cancer cell line, MDA-MB-231, with either a high or low metastatic propensity to the bone. Gene array comparison of these cell types has resulted in the isolation of a number of candidate molecules whose expression correlates either positively or negatively with bone metastasis. We are currently using a variety of molecular and cellular approaches to characterize these genes, to determine their role in cancer cell biology, metastasis and their prognostic significance in human breast cancer. Through this work it is hoped that new therapeutic targets and novel prognostic markers will be identified in breast cancer.

**Hsp90 Inhibitors.** The molecular chaperone, Hsp90, regulates over 100 proteins within the cell, a number of which are central to cancer cell growth and progression. As a result, Hsp90 has emerged as an important clinical target in cancer research. The ansamycin antibiotics, such as geldanamycin and herbimycin A are known to inhibit Hsp90 function and derivative compounds, such as 17 allylamino-17-desmethoxygeldanamycin (17-AAG), have successfully progressed to stage II patient clinical trials. In preclinical models, although we have shown that 17-AAG has potent anti-tumour activity in the mammary-fat pad, in the bone microenvironment however, 17-AAG can promote tumour growth. We are currently investigating the molecular mechanisms by which 17-AAG can enhance bone degradation. To better understand the actions of HSP90 inhibitors, we have recently generated human breast cancer cell lines resistant to 17-AAG and are currently characterizing these at the molecular and cellular levels.

**IGFBP-2 and the αvβ3 Integrin Interaction in Cancer Growth and Progression.**

Our laboratory has identified a novel bi-molecular interaction between the cell surface molecule, αvβ3 integrin and the insulin-like growth factor binding protein 2 (IGFBP-2). Both of these molecules have been independently shown to correlate with poor prognosis and progression in a number of cancers, including prostate, glioma and neuroblastoma. Therefore, we are investigating if the specific interaction of IGFBP-2 and/or its proteolytic fragments with the αvβ3 integrin represents a novel pathway in prostate and glioma tumour progression and metastasis.

Localization of IGFBP-2 (red), αvβ3 (green) and their co-localization (yellow) in human breast cancer cell line, MCF-7/β3, mammary-fat pad tumours.
A cell’s ability to respond to its extracellular environment involves a complex & highly organised series of events referred to as cellular signalling. Our laboratory focuses on a group of enzymes known as the Protein Tyrosine Phosphatases (PTPs) that regulate tyrosine phosphorylation-dependent cellular signalling. We use cutting-edge biochemical, cell biological & imaging approaches as well as knockout mice & Drosophila genetics to delineate the roles of PTPs in varied human diseases. Honours projects available in 2013 include the following:

**OBESITY & TYPE 2 DIABETES RESEARCH**

**Molecular Mechanisms of Central Leptin Resistance & Obesity**

*Supervisors: Prof T. Tiganis & Prof M. Cowley*

Leptin is a hormone that is produced by white adipose tissue & acts centrally to integrate the status of peripheral energy stores with the central adaptive control of energy expenditure. Leptin signals in the hypothalamus to decrease food intake & increase energy expenditure.

Leptin signals via the JAK2 protein tyrosine kinase that promotes signalling via a variety of effector cascades, including the Ras/MAPK, PI3K/Akt & STAT3 pathways.

The anorectic & metabolic responses to leptin are diminished in obese rodents & humans & this is thought to be a key contributing factor to the development of diet-induced obesity. Several underlying mechanisms are thought to contribute to the decreased leptin responsiveness including alterations in the blood brain barrier, gliosis, as well as neuronal cell-intrinsic mechanisms that directly attenuate leptin signalling. Elevated hypothalamic PTP1B & SOCS3 levels in diet-induced obesity contribute to the attenuation of the leptin signal. Recently our laboratory established the role of the phosphatase TCPTP in central control of energy balance & established that elevated hypothalamic TGPTP levels contribute to the development of cellular leptin resistance & obesity [Loh et al. (2011) Cell Met 14, 284-99]. Projects are available to assess the neuronal cell-specific contributions of PTP1B, SOCS3 & TCPTP to the development of cellular leptin resistance & obesity.

**Type 2 Diabetes & the Molecular Basis of Selective Insulin Resistance**

*Supervisor: Prof. T. Tiganis & Dr. Esteban Gurzov*

Obesity is increasing at an alarming rate worldwide & is a major risk factor for type 2 diabetes. Obesity & in particular increased visceral (abdominal) adiposity, is causally linked to the development of insulin resistance, a major hallmark of type 2 diabetes. Insulin resistance refers to the state of diminished insulin responsiveness in insulin’s key target tissues.

Insulin acts co-ordinately in liver, muscle, fat & hypothalamus to lower blood glucose levels after a meal. Insulin signals via the insulin receptor (IR) protein tyrosine kinase, which in turn activates the PI3K/Akt pathway to mediate insulin’s metabolic effects. Insulin resistance is associated with defects or aberrations in signalling downstream of the IR, in particular in the PI3K pathway.

It is important to note that the defect in insulin signalling in type 2 diabetes does not occur in all pathways. In particular, it is well established that insulin resistance differentially impacts on hepatic glucose versus lipid production; whereas the insulin-induced suppression of gluconeogenesis is defective in type 2 diabetes, insulin’s ability to induce lipogenesis remains intact, contributing to the development of hepatic steatosis & the vascular complications associated with diabetes. Projects are available to delineate the molecular mechanisms of selective insulin resistance.

**Reactive Oxygen Species & Insulin Sensitivity**

*Supervisor: Prof. T. Tiganis*

Oxidative stress, or the chronic generation of reactive oxygen species (ROS), is thought to contribute to the progression of various human diseases including type 2 diabetes. In type 2 diabetes, ROS are thought to promote insulin resistance.

Although the excessive production of ROS by mitochondria is detrimental, paradoxically, ROS generated by NADP(H) oxidases at the plasma membrane/endomembranes may be required for normal intracellular signaling. A wide variety of stimuli including insulin can promote the transient generation of ROS. PTPs are key targets of such ‘physiological’ ROS. We have shown previously that ROS may promote insulin sensitivity early in disease onset via the oxidation & inhibition of PTPs. A project aimed at delineating the physiological versus pathological contributions of ROS to type 2 diabetes is available.
IMMUNOLOGY RESEARCH

TCPTP & Autoimmunity

Prof. T. Tiganis & Dr. Florian Wiede.
Autoimmune diseases collectively affect ~5% of individuals world-wide. Inflammatory bowel disease (IBD), which includes Crohns & ulcerative colitis, affects > 60,000 people in Australia with a total financial burden of 2.7 billion. Type 1 diabetes affects > 120,000 Australians & is the fastest growing chronic childhood disease increasing by 3% p.a. & costing > 3 billion/year.

Autoimmune diseases are polygenic & are influenced by environment. Genome wide association (GWA) studies have linked SNPs (single nucleotide polymorphisms) in the PTPN2 gene encoding the protein tyrosine phosphatase TCPTP with the development of IBD & type 1diabetes, whereas our own recent studies have shown that TCPTP deficiency in T cells [Wiede et al (2011) J Clin Invest 121(12):4758-74] results in spontaneous autoimmunity. Projects are available to define TCPTP’s role in the establishment of peripheral T cell tolerance & the prevention of autoimmunity.

Differentiating Self- from Non-Self

Prof. T. Tiganis & Dr. Florian Wiede.

T cells play a central role in the adaptive immune response continuously surveying & responding to cellular abnormalities & pathogens/infections. T cells are generated by the thymus largely in early life & then maintained throughout adult life by complex homeostatic mechanisms. Both T cell homeostasis & pathogen-induced T cell activation are reliant on T cells engaging & responding to self- & foreign-peptide antigens respectively via cell surface T cell receptors (TCRs). The resultant level of TCR signalling is key to ensuring that the responses to high affinity foreign antigens are robust & that pathogens & infected cells are eliminated effectively, whereas responses to lower affinity self-antigens are restricted, allowing for T cell survival & cell division, whilst preventing excessive responses & the development of autoimmunity.

TCR proximal signalling, mediated by Src family protein tyrosine kinases (SFKs), is essential for both T cell homeostasis & immunity. The precise intracellular mechanisms by which T cells differentially ‘sense’ & respond to ‘low’ affinity self- v/s high affinity foreign-antigens & in particular, the mechanisms by which SFKs are regulated, remain unclear. Projects are available to determine the role of PTPs in tuning TCR/SFK signalling & responses to self- versus foreign-antigens.

CANCER RESEARCH

TCPTP & Breast Cancer

Supervisor: Prof. T. Tiganis.

Breast cancer is the most frequent malignancy among women, with an estimated one million new cases per year worldwide. The majority of breast cancers can be classified into those that are estrogen receptor positive & respond to anti-estrogen & those that are ER negative, but express members of the epidermal growth factor receptor (EGFR) family protein tyrosine kinases. In either case, tyrosine phosphorylation-dependent signaling, as mediated by EGFRs, or for example SFKs that propagate ER & EGFR signalling, is critical to the development & progression of breast cancer.

Our laboratory has shown that EGFRs & SFKs can serve as substrates for the PTP known as TCPTP. Furthermore, we have shown that TCPTP attenuates the tumorigenicity that is associated with the overexpression/activation of EGFRs &/or SFKs in tumour cells & the genomic instability that can be associated with PTK hyperactivation. Our preliminary studies indicate that TCPTP expression is reduced in a large number of breast cancer cell lines in vitro & breast cancers in vivo. We hypothesise that TCPTP may serve as a tumour suppressor in breast cancer. A project is available to characterise TCPTP’s role in breast cancer development.

Obesity & Liver Cancer

Supervisor: Prof. T. Tiganis & Dr. Esteban Gurzov

Primary liver cancer is one of the world’s deadliest cancers. It is the 5th most common cancer worldwide & represents the 3rd most common cause of cancer death. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers & is refractory to nearly all currently available anti-cancer therapies with a 5 yr survival rate of < 9%. HCC is most common in less developed nations, where it is typically associated with chronic hepatitis B. However, over the last 20 years, the incidence of HCC in more economically developed countries has been increasing: HCC has almost doubled in the US & nearly tripled in Australia in the last 20 years. The obesity epidemic is thought to account for as much as 50% of the increase in HCC in developed nations.

Obesity & type 2 diabetes are linked to the development of liver steatosis, which can progress to steatohepatitis & pericellular fibrosis to cirrhosis & HCC. There are ~1.6 billion overweight adults worldwide of whom > 400 million are obese (BMI>30) & this is predicted to rise to 2.3 billion by 2015 (WHO) & to be largely unabated by lifestyle intervention. The prevalence & increasing trend of obesity/diabetes in the developed world will undoubtedly translate into a significant rise in HCC incidence in the years to come. Projects are available to delineate the molecular mechanisms by which obesity contributes to the development of HCC.
2. Education Research:

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The research conducted by members of the Biochemistry Education Research Team focuses on student learning in Biochemistry and Molecular Biology, Health and Medical Education. The overall aim is to improve the learning and teaching process and student learning outcomes. The group uses both quantitative and qualitative research methods to provide evidenced-based best practice. All members of the team are Education Focused Academics with research backgrounds in Biochemistry and Molecular Biology. Current areas of research include:

**Student engagement: measures, motivation and academic impact**

- A collaborative study is underway to determine the extent of Monash University students engagement with lectures and their studies in general. The study aims to evaluate students’ reasons for non-attendance, participation and lack of engagement with a goal to enable staff to examine and redesign the learning modes and resources used.

- A project examining the study habits of undergraduate students is about to commence. This project will examine study habits looking for correlations with student results and the ways in which we can increase student engagement with their studies.

**Curriculum design and implementation**

- Projects are involved in the design, implementation and evaluation of innovative learning activities and their impact on student learning and intellectual stimulation. In particular projects designing and evaluating new laboratory classes and associated activities will be undertaken.

- A study of the impact of prior learning experiences on student academic progress is underway. The aim of this research project is to determine if the pre-requisites studies currently required for Biochemistry are impacting on the academic success of students studying Biochemistry at Monash University. This information will then inform the amendment of pre-requisite requirements, student support and teaching.

**Assessment: the affect of assessment practices on student’s learning**

Assessment of learning is a critical component of education. Assessment in medical education has been the subject of rigorous study to ensure accurate and reliable assessment practices with which to determine student’s readiness for progression. Less rigorous approaches to assessment have been used in other disciplines. We aim to design, implement and evaluate high quality assessment practices which are reliable and equitable and assess higher order learning. We are particularly interested in examining the use of online quizzes (summative or formative) and the final results achieved by the students and also the value of peer assessment for students with respect to the skills they can learn from being both the assessors and those being assessed.

**Creativity in Education**

Creative thinking can lead to new insights and novel approaches to problems and is one of the key skills many prospective employers are looking for. However, the science curriculum can be overwhelming in terms of discipline content knowledge and fail to give students the opportunity to be truly creative. This project will examine ways in which we can encourage students to retain and develop their curiosity and creativity.

**The Teaching-Research nexus; how research informs and enhances learning**

The teaching and research nexus describes the relationship between teaching and research within a university. There is a rationale that research forms the knowledge base for teaching by introducing current topics and new methodologies, however empirical evidence for a relationship between research performance and the quality of teaching is not strong. Despite this many scholars argue that teaching and research are inextricably linked for mutual benefit, and can benefit student learning and improve graduate attributes. Student awareness of research is a critical factor in the integration of research and teaching. Most students feel there is a relationship but the way they perceive this varies according to the nature of the discipline. There is a need for more studies on how the nexus impacts on student engagement in specific disciplines. The proposed study will evaluate the extent of the teaching-research nexus in the discipline of Biochemistry at Monash University through the eyes of our students, to identify the benefits of the nexus to student learning.
3. Genetics & Development:

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A critical control of gene expression is the turning “on” and “off” of mRNA transcription by DNA binding proteins. A second essential, but far less-well appreciated level of control is that which determines when, where and for how long the resulting mRNA transcripts are translated into protein. We are interested in this post-transcriptional control of gene expression. Our current areas of studies are:

- The regulation of fat-metabolism within the circadian rhythm using zebrafish and mouse models.
- The control of translation by 3’UTR elements in the model yeast *Saccharomyces cerevisiae* and in tissue-culture cells.
- The roles that non-coding RNA play in shaping gene expression.
- Systems Biology: Computational integration of genome-wide “omic” datasets to build useful network/pathway models in cell biology (with Dr. Jonathan Keith from the Mathematical Sciences)

Research projects suitable for students at the Honours and PhD level are available and can be tailored to the interests of applicants. We use a wide range of molecular biology tools with a particular focus on RNA biology applying high-content/high through-put technologies. Students with a background and/or interest in bioinformatics are also encouraged to apply.
Accurate temporal and spatial regulation of mRNA translation and degradation is critical for control of gene expression. A dramatic example of posttranscriptional regulation occurs during formation of oocytes (oogenesis) where thousands of mRNAs are transcribed prior to the characteristic shutdown of transcription late in oogenesis (prophase of meiosis I). A large proportion of these mRNAs are maintained in a translationally silent state until specific stimuli initiate their translation, facilitating the completion of oocyte development, fertilisation and early embryonic development – all in an environment devoid of transcription. Interestingly, very recent reports suggest that proteins similar to those that regulate some mRNAs during oogenesis are also involved in mRNA localisation and translational regulation in neurons. In both these cellular contexts, there is a critical need to temporally and spatially control mRNA translation, however the broad regulatory pathways governing this process are almost entirely mysterious.

Various mechanisms that repress translation and degrade mRNAs are concentrated in cytoplasmic structures known as Processing bodies (P bodies), which include decapping- and nonsense-mediated mRNA degradation, and small RNA-mediated translational silencing and mRNA decay. We use oogenesis in C. elegans (round worm) as a model system to study posttranscriptional gene regulation and have identified a conserved RNA-protein complex that shares many components with P bodies. This RNA-protein complex associates with a specific subset of gonad mRNAs and is required for their stability and translational repression; accordingly, we have called these structures Storage bodies.

- **Storage bodies**: sites containing translationally inhibited and protected mRNAs
- **P bodies**: primarily sites of mRNA degradation via multiple pathways

The C. elegans gonad expresses ~6000 genes during oogenesis. Of these only a small proportion specifically associate with the Storage body protein CGH-1, a DEAD box RNA helicase, suggesting the existence of a discrete pathway for their recruitment. A major focus of the lab will be to elucidate the mechanisms that regulate the recruitment of mRNAs to Storage bodies. A sub-set of the CGH-1 associated mRNAs will be used as molecular markers for analysis of recruitment of mRNAs to Storage bodies using immunolocalisation techniques in various mutant or RNA interference (RNAi) backgrounds for Storage body related proteins. In many systems mRNA translation and stability are regulated by sequence-specific elements in the 3’ untranslated region (3’ UTR). Thus, one approach to identifying the signals that target mRNAs to storage bodies will involve bioinformatic analysis of the 3’ UTRs of CGH-1 associated mRNAs for enrichment of specific sequence motifs. However, it is possible that the Storage body localisation signal will not be contained in a simple linear sequence motif. Therefore we will also pursue complimentary approaches such as, mutational analysis of specific 3’UTRs (random or targeted to conserved sequences) for their ability to regulate mRNA localisation and translation of a reporter gene transcribed by a germline-specific promoter. We are also interested in identifying and characterising additional Storage body proteins. Harnessing the power of RNAi in C. elegans, we will undertake a high throughput screen for genes that interact with the CGH-1 RNA-protein complex and cause sterility.

Together these studies will provide valuable information regarding mechanisms of mRNA localisation and translational silencing during reproduction in metazoan species. Given the apparent ubiquitous nature of P body components, these studies will provide a framework in which to think about Storage body function in other cellular contexts.

**Translation and degradation of Storage body associated mRNAs**

The translation of maternally supplied mRNAs in the early embryo is essential for development to occur. How mRNAs are released from their storage/translational repressive states remains a fundamental question to be addressed in metazoan development. The C. elegans system is particularly well suited to pursue this question, as the worm is self-fertilising, allowing oocyte maturation, fertilisation and embryogenesis to be visualised in an intact animal. Coupling RNAi and targeted mutagenesis of fluorescently tagged Storage body proteins, for example CGH-1::GFP, will allow real-time visualisation of the dynamics of Storage bodies (including movement and protein degradation) during this important developmental period. Developing a molecular understanding of the formation of Storage bodies and P bodies during early embryogenesis may reveal additional regulatory pathways required for development, and it is likely that similar events occur more broadly in metazoan development.
Investigating crosstalk between testosterone and the activin signalling pathway: mechanisms, consequences and physiological relevance

**Research Group Name:** Testis Development and Germ Cell Differentiation Laboratory  
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**Project Description**
Sertoli cells are somatic cells within the testis that translate hormonal signals to create the unique microenvironment required to maintain the spermatogonial stem cell population and support sperm production. Our ongoing studies address the requirement for precisely regulated crosstalk between the growth factor activin A and the hormone testosterone. We recently made the unexpected discovery that testosterone can activate the activin signalling molecule Smad3. This project will explore the mechanism underpinning this transactivation and determine the effect on Smad3 signalling when testosterone activity is blocked. Students will gain skills in tissue culture, quantification of signal transduction, imaging and automated image analysis (brightfield, fluorescence and confocal microscopy), gene expression analyses (quantitative RTPCR, Western blot) and histology using wildtype mice and a genetically modified mouse tumour model. This project will uncover important new insights for understanding how activin and hormones cross-talk during development and in diseases such as cancer, ischemic/reperfusion injury and fibrosis.
The Molecular Genetics of Down Syndrome
Dr. Melanie Pritchard  
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Down syndrome (DS) is the most common chromosomal abnormality and is the most common cause of intellectual disability due to developmental abnormalities of the brain. It is also associated with abnormalities in most other major organ systems of the body, including the skeleton, heart, gastrointestinal tract, vision and hearing. People with DS are prone to infections due to immune system deficits and to certain types of autoimmune disease and cancer. In addition, all individuals with DS develop Alzheimer's-like pathology in the brain by the age of 35. Notably, many of the problems experienced by people with DS also occur in the rest of the population - albeit at a lower frequency and later in life. Therefore, DS is very useful model for common disorders and what we learn from DS is applicable to the broader community.

We use the mouse as a model for human genetic disease and have focussed our studies on genes that are likely disease candidates. In order to determine the functions of these genes and to investigate the consequences of their over-expression we have generated knockout mice (to determine the normal biological function of the gene) and transgenic mice, where the gene of interest is over-expressed to mimic the situation in DS. Our group is primarily interested in understanding how these genes function in the brain. We have identified and are currently working on two novel chromosome 21 genes, DSCR1/RCAN1 and Intersectin-1 (ITSN1). Both are involved in neuronal function and have the potential to contribute to the brain anomalies characteristic of the DS phenotype, including Alzheimer’s disease.

Techniques to be used: immunohistochemistry, immunocytochemistry, primary neuronal cell culture, immunoblotting.

Honours and PhD projects exist in these areas (see also Dr A Lawen), and will combine the use of knockout and transgenic mice with cellular assay systems to elucidate the cellular mechanisms and signal transduction pathways involved.
Cutaneous Developmental Biology Lab

Dr Ian Smyth
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Projects:
The contribution of the Fras and Frem proteins to Pdgf signalling and cleft palate
Development and disease progression are shaped by the activity of growth factors whose presence can direct cells to differentiate or divide. A key mediator of this effect is the basement membrane, a proteinaceous structure which underlies most embryonic and adult epithelia. The project focuses on the role of a family of genes which encode the Fras and Frem basement membrane proteins. Loss of these genes leads to an array of developmental and adult defects affecting the skin and other organs. This project will explore a recently identified role for these proteins in controlling the activity of growth factors in the extracellular environment, particularly those of the Pdgf family. We will specifically investigate how the ability of these proteins to shape Pdgf signalling leads to defects in palate development which result in facial clefting. Cleft lip and palate are one of the most common developmental defects affecting ~1/500 newborns.

How and where do nephrons form during kidney development
Nephrons are the functional units of the kidney but we have a limited understanding about where they form spatially during kidney development, relative to the branching ureteric tree which drives organ development. This project will employ an imaging technique known as Optical Projection Tomography to visualise, in 3 dimensions, different stages of nephron development over embryonic time. We will then quantify this spatially relative to the whole organ and study nephron formation in different models of abnormal development and disease.

The role of the Fras and Frem proteins in the development of congenital diaphragmatic hernia (CDH)
Congenital diaphragmatic hernia (CDH) is a poorly understood birth defect in which the diaphragm fails to normally separate the chest and abdominal cavities. As a consequence the abdominal contents (stomach, liver, spleen, intestines) can be displaced into the chest cavity. This can severely impact on the development of the lungs and heart resulting in developmental delay and defects in the formation of these organs. We have recently shown that mutations in members of the FRAS and FREM family of extracellular matrix proteins can lead to this developmental defect. This project will investigate the developmental basis of this defect and examine at molecular and cellular levels, the consequences of loss of one particular protein, Frem2, in diaphragm development. By investigating the role of these proteins in the development of the diaphragm we hope to gain better insights into the genetic and mechanistic bases of this defect.

Understanding the role of inflammation in Harlequin Ichthyosis
Harlequin Ichthyosis (HI) is a devastating skin disease caused by mutations in ABCA12, a gene necessary for normal lipid transport in the skin. We have shown that loss of this gene results in the initiation of an inflammatory response in the developing skin prior to birth. This project will investigate the nature of this defect and how it contributes to disease in HI patients. Using immunohistochemistry and quantitative RT-PCR we will examine the expression of inflammatory cytokines in a model of this disease. We will also utilise a transgenic mouse expressing an immune suppressor to gauge how pro-inflammatory molecules give rise to disease in HI patients and the extent to which modulating this response might be used as a therapeutic approach to correct disease features in affected patients.
Epigenetics and Chromatin Research Group
Dr. Lee Wong
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Our entire genome, an estimated 60 million base pairs, when unwound, would measure 2 metres in length from end to end – yet it is packaged to fit within a nucleus no larger that 2uM in diameter. DNA is wrapped around an octameric core of proteins called histones. This complex of DNA and proteins is called chromatin, which folds back upon itself several times to form higher magnitudes of compaction. At the same time, the chromatin must still be accessible to allow vital processes such as gene expression and DNA replication to take place; and this is largely regulated by epigenetic mechanisms, which include DNA methylation and histone modifications.

Centromeres and telomeres are specialised structures found at the centre and ends of all eukaryotic chromosomes, respectively. Our aim is to define how epigenetic factors organise the chromatin structure of centromeres and telomeres in stem cells and cancers. Our research will impact the understanding of fundamental epigenetic mechanisms that regulate chromatin states, and their contribution towards the control of stem cell pluripotency, cellular differentiation and the clinical progression in certain diseased conditions including cancers.

Epigenetic regulation of telomere chromatin in stem cells and cancers
Telomeres are structures that protect the chromosome ends. In human cells, the telomere DNA repeat is lost at each cell division. This progressive shortening of telomere limits the proliferative potential of somatic cells. About 85% of human tumours have acquired an indefinite replicative capacity by increasing telomerase expression, however, of the remaining 15%, most are able to maintain their telomere length in the absence of telomerase by a DNA recombination-mediated mechanism known as the Alternative lengthening of telomeres (ALT). ALT mechanism is utilized in a variety of human cancers including osteosarcomas, liposarcomas, glioblastoma and astrocytomas.

Honours Project 1: Histone H3.3 and the regulation of chromatin in human ALT cancers
In mammalian cells, the repressive chromatin marks including DNA hypermethylation, histone H3 lysine 9 tri-methylation (H3K9me3) and histone H4 lysine 20 tri-methylation (H4K20me3) are critical for the formation of a compact telomeric structure, negative regulation of telomere length and repression of telomeric DNA recombination. Much remains unknown about the epigenetic factors that control telomere function in pluripotent embryonic stem (ES) cells. We have shown that histone variant H3.3 (Fig. 1) and chromatin remodeler ATRX (alpha-thalassemia mental retardation) play a unique role in maintaining telomeric chromatin integrity in mouse embryonic stem cells. We hypothesize that this ATRX/H3.3-mediated telomeric chromatin maintenance has an impact on the formation of a compact structure for protection and repression of recombination of the telomeres in mouse embryonic stem (ES) cells.

![Fig. 1 Staining of phosphorylated histone H3.3 (green) at telomeres (arrows) in pluripotent mouse embryonic stem cells during mitosis.](image)

Honours Project 1: Histone H3.3 and the regulation of chromatin in human ALT cancer cells
In ES cells, Histone H3.3 serine 31 phosphorylation (pH3.3) is found at the telomeres, while in somatic cells, pH3.3 is found only at the pericentromeric regions of mitotic chromosomes. Interestingly, ALT cancer cells frequently carry mutations in the H3.3 chaperones ATRX and Daxx. Given that pH3.3 has been implicated in the maintenance of telomere chromatin in ES cells, it would be interesting to investigate the dynamics of pH3.3 in ALT cancer cells. Using a panel of ALT cancer patient cell lines, the preliminary evidence suggests that H3.3 regulation is perturbed in ALT cells. It would be interesting to investigate the...
relationship between ATRX, Daxx and H3.3 in the regulation of telomeres and general chromatin of ALT cancer cells. This project will help to define how mis-regulation of H3.3 chromatin modification could contribute to cellular immortality in ALT cancers.

Techniques: cell culture, immunofluorescence, Western blotting, RNAi knockdown, DNA sequencing, chromatin analysis and real-time PCR.

Honours Project 2: The role of a novel histone kinase at the telomeres of embryonic stem cells.
Histone H3.3 serine 31 phosphorylation (pH3.3) is a unique epigenetic mark found only at centromeric regions during mitosis in somatic mammalian cells and telomeres of ES cells. Although pH3.3 has been described in the literature, the kinase responsible for H3.3 serine 31 phosphorylation is has remained elusive. This is particular interesting since pH3.3 has been implicated in the maintenance of ES cell telomere chromatin. This project will focus on testing previously identified candidate proteins, some of which are novel telomere proteins, as potential H3.3 serine 31 kinases. We will also investigate the impact of pH3.3 upon transcription and chromatin structure at these regions. This is particularly important since pH3.3 has been shown to be important in the maintenance of ES cell telomere chromatin. Techniques: ES cell culture, immunofluorescence, Western blotting, RNAi knockdown, chromatin analysis, FISH (fluorescence in situ hybridization), in vitro kinase assay and real-time PCR.

Honours Project 3: Epigenetic regulation of centromere chromatin in cancers.
Although canonical centromeres are heterochromatic and filled with repetitive sequences, recent studies have shown that centromeres are transcriptionally competent. We have showed that non-coding RNA (ncRNA) is required for controlling proper chromosome segregation during mitosis and it acts as a novel epigenetic determinant of centromere function. The centromere chromatin is defined by the presence of CenpA (centromeric specific histone H3). One of our aims is to investigate the role of ncRNA transcription in the perpetuation of centromere chromatin identity- through the recruitment of CenpA chromatin assembly complex, and how this process may be disrupted in cancer cells. This study will unveil how centromere defects contribute to chromosomal abnormalities and tumorigenesis. Techniques: ES cell culture, immunofluorescence, Western blotting, RNAi knockdown, FISH (fluorescence in situ hybridization), chromatin analysis in vitro kinase assay, real-time PCR.

Selected publications


4. Infection & Immunity

Viral immune Evasion
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We aim to understand the structure and function of proteins involved in anti-viral immunity

Viral infection is a significant cause of global mortality and economic burden. Did you know the Spanish influenza outbreak of 1918 killed more than 40 million people? Or that hepatitis B is the most common infectious disease in the world, causing 600,000 deaths each year? The success of a virus is partly determined by how well it can evade the host innate immune response. Immune evasion strategies are implemented by well-known RNA viruses like hepatitis A, hepatitis C, SARS, influenza A, measles, mumps and the Hendra virus. Unfortunately however, these immune evasion mechanisms are often poorly understood and hampered by our limited understanding of how anti-viral immunity is activated by the RIG-I like receptors following infection with an RNA virus. Using a combination of functional and structural studies, our aim is to understand how anti-viral immunity is modulated by host and viral proteins. This knowledge may be exploited to design anti-viral therapeutics and vaccines that can enhance the immune response towards RNA viruses or to directly counteract them.

PROJECT AREAS:
1) Influenza virus and the Hendra virus adopt a multi-pronged approach to immune evasion. We’re interested in understanding precisely how viral proteins hijack host proteins to enhance virus survival.

2) E3 ligases and deubiquitinases are enzymes that attach/remove ubiquitin to/from pre-existing proteins. The attachment/removal of ubiquitin serves as a signal to the cell and can alter the function, localization or binding partner of the protein. Despite its significance, ubiquitin modification is vastly under-studied in Australia. Numerous ubiquitin-modifying enzymes exist in innate immune signalling pathways, and no doubt many are yet to be identified. We are interested in identifying novel substrates of these enzymes to better understand how these signalling pathways are regulated.

TECHNIQUES:
Techniques used within the laboratory include molecular biology, recombinant protein expression using insect and bacterial cells, protein purification using a Fast Protein Liquid Chromatography system, X-ray crystallography, Small Angle X-ray Scattering, molecular dynamics, protein-protein interaction analysis (eg. surface plasmon resonance, enzyme assays, ITC). Cellular assays are also used to characterize gene expression, regulation and localization and to study our innate immune response to viral infection.
Microbial Oncogenesis Laboratory

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At least 15% of malignancies worldwide are attributed to chronic infections by viruses, bacteria, and other pathogens. The molecular mechanisms underlying Helicobacter pylori (Hp)-induced gastric cancer and chronic gastritis are poorly understood. Our laboratory uses Helicobacter pylori infection as a model system for identifying host factors and signalling pathways involved in chronic inflammation and carcinogenesis.

Project 1: Structure and functions of the Hp virulence protein CagL
Aim: We have recently identified a surface protein of Hp, CagL, which interacts with integrin receptors on human stomach epithelial cells. The aim of this project is to determine the 3D-structure of CagL and identify amino acids in CagL important for determining cancer risk and disease outcomes. The work may open up new avenues for development of novel drugs against Hp infections.
Techniques: Molecular cloning, in vitro mutagenesis, protein purification, binding assays, circular dichroism, X-ray crystallography and/or NMR

Project 2: Identification and characterisation of integrin receptors involved in Hp-induced chronic inflammation and oncogenesis
Aim: Integrins comprise an important family of eukaryotic receptors with key roles in cell adhesion, migration, inflammatory responses and carcinogenesis. The aim of this project is to characterise the expression and functional roles of integrins in Hp-mediated inflammation and cancer.
Techniques: Gene knockdown by RNAi, mammalian cell culture, transfection, live cell imaging, immunofluorescence microscopy, Western blotting, immunoprecipitation and cloning

Project 3: Signal transduction pathways triggered by integrin-Hp interaction
Aim: Some virulence proteins of Hp, by interacting with integrins and other mammalian receptors, significantly perturb normal signal transduction pathways in the host cell. The aim of this project is to understand the molecular mechanisms by which Hp virulence proteins interfere with integrin-mediated signal transduction pathways and how that might lead to cellular transformation.
Techniques: Gene knockdown by RNAi, pull-down assays, Western blotting, immunoprecipitation, kinase assays and live cell imaging.
How does the type II secretion system recognize its deadly substrates?

Prof. Trevor Lithgow & Dr. Matthew Belousoff & Dr. Eva Heinz.

Bacterial outer membranes incorporate proteins of at least three well-characterized architectures: β-barrel proteins, lipoproteins and secretins. Secretins are integral proteins which assemble to form dodecameric secretion channels in the outer membrane, with examples including outer membrane proteins of the Type II Secretion Systems (T2SS) as well as Type III Secretion Systems, Type IV fimbriae and the filamentous phage extrusion machinery. In the case of the T2SS, the outer membrane secretin dodecamer docks onto a platform of proteins in the inner membrane that energize its function in the selection and secretion of a very specific set of substrate proteins out into the external milieu.

In *Vibrio cholerae*, for example, the T2SS mediates the secretion of cholera toxin and as many as 20 other substrates. Substrate recognition by the T2SS is a real mystery, but recent work suggests that substrates are directly recognized by the secretin. Bioinformatics we have recently completed shows that at least three classes of secretins exist and, therefore, experiments to define the targeting sequences recognized by the T2SS need to use both substrates and secretins from a single type. We are working with the *Vibrio*-type secretin which is found in the T2SS in species of *Vibrio* and *Shigella* and a few pathotypes of *E. coli* (our favourite of these is enteropathogenic *E. coli*, or EPEC).

We know that bacteria expressing a *Vibrio*-type secretin can recognize and secrete cholera toxin and the related heat-labile toxin. Bacteria expressing the *Klebsiella*-type or *Pseudomonas*-type secretins apparently cannot. The hypothesis then would be that *Vibrio*-type secretins can specifically recognize cholera toxin and other substrates secreted by *Vibrio*, *Shigella* and EPEC. But how?

Several hypotheses will be tested. This project will provide training in bioinformatics, recombinant DNA methods: plasmid construction, mutagenesis, DNA sequencing, protein expression and characterization, and use of protein transport assays recently developed in our laboratory.
Molecular Immunology and Immunoproteomics

Professor Tony Purcell

The Purcell Laboratory is internationally renowned for research into the identification of targets of the immune response in infectious disease and autoimmunity. Having recently relocated to Monash we have established a state of the art molecular immunology and proteomics laboratory. Our laboratory uses advanced mass spectrometry to discover new T and B cell epitopes, to study host-pathogen interactions and to quantitate antigen presentation in a number of human diseases. We work closely with other laboratories to understand immune recognition at a molecular level. We have two major goals; (i) to develop highly specific drugs or immunotherapeutics to enhance immunity to human pathogens including Malaria, Hendra Virus, influenza and Epstein barr virus and (ii) To design drugs or immunotherapeutics to ameliorate immune responses to normal tissues in autoimmune diseases such as type 1 diabetes, arthritis and multiple sclerosis.

Project areas

Bat vs Man

Hendra virus (HeV) is an emerging zoonotic pathogen that while non-symptomatic in the natural fruit bat host, is characterised by high morbidity and mortality in horses and humans. Although no direct spread of virus from the bat host to humans or between humans has been reported, the potential for the virus to mutate and take these infective pathways is a serious concern. The immune response of the natural host may provide the key to deciphering the elements of an effective anti-viral strategy. We hypothesise that bats control HeV through a combination of a robust innate immune response to the virus, combined with the action of an adaptive T-cell mediated immune response. The adaptive immune response involves T cell recognition of viral peptides complexed to molecules encoded by the Major Histocompatibility Complex (MHC). Adaptive immunity elicits cytotoxic responses that kill virally infected cells and also antibodies capable of neutralising the virus. We wish to examine the adaptive immune response in bats and humans using state-of the art mass spectrometry techniques. We aim to characterise the MHC-bound peptides selected for presentation in both humans and bats to gain insights into potential targets for rational drug design, vaccine development and clinical monitoring.

A Pox on you! Understanding the dynamics of viral antigen presentation

CD8+ T cells eliminate virus-infected cells following recognition of viral peptides (epitopes) bound to MHC molecules on the surface of infected cells, by their T cell receptor (TCR). A single cell may display hundreds of thousands of different MHC-peptide complexes, many derived from endogenous proteins, yet our understanding of the proportion of viral epitopes presented on the cell surface and how constitutive antigen presentation is altered is poorly understood. This project combines mass spectrometry and proteomics techniques with virology and immunology to address how viruses alter the peptides displayed on the surface of infected cells using vaccinia virus (VACV) as a model. Work already undertaken has quantified a small subset of VACV epitopes during infection of murine cells, but many epitopes remain to be determined and studied (Figure 2). These include not just viral epitopes, but also novel host cell peptides presented during infection, both of which could be used to stimulate anti-viral immunity.

A prospective student would become highly skilled in core aspects of mass spectrometry, liquid chromatography, protein purification, MHC capture and epitope elution as well as learning essential data validation and analysis techniques.
**Sweet targets! Identification of novel T-cell targets in diabetes.**

In type 1 diabetes (T1D), the immune system mistakenly recognises peptides derived from self-proteins, triggering an attack against the insulin secreting β cells, leading to β cell loss and insulin deficiency. T cell recognition of self-peptides complexed to molecules encoded by the Major Histocompatibility Complex (MHC) is involved in both the initiation and progression of disease. Identifying the peptides targeted during diabetes development thus remains a critical step in defining the molecular basis of this disease. We have shown that known diabetogenic T cell epitopes may be displayed on the surface of β cells in very low abundance. This complicates the identification of novel T-cell epitopes by mass spectrometry, which is biased towards identifying peptides of high abundance. We have developed methods to specifically target candidate autoantigens for degradation by the proteasome, thereby increasing the peptide pool available for binding to MHC. This approach will provide a novel panel of potential T-cell targets and will allow the identification of specific post-translational modifications. Presentation of post-translationally modified peptides is proposed to be a key player in the development of immunity towards β cells, which are exposed to an increasingly hostile inflammatory environment during progression to overt diabetes.

**Drugs, bugs and immunity**

Because of their central role in immunity HLA alleles are often associated with positive and negative outcomes in infectious disease, autoimmunity and cancer. In addition, a growing number of immunologically-based drug reactions are also associated with specific HLA alleles. We have recently defined the underlying mechanisms of these associations, including abacavir hypersensitivity syndrome (AHS), a prototypical HLA-associated drug reaction. AHS occurs in individuals with the common histocompatibility molecule, *HLA-B*<sup>*</sup>57:01 (relative risk >1000). We have shown that abacavir binds non-covalently to HLA-B*57:01 changing the shape and chemistry of the Ag-binding cleft, thereby altering the repertoire of endogenous peptides that can bind HLA-B*57:01. In this way, abacavir guides selection of new endogenous peptides, inducing a dramatic alteration in ‘immunological self’. The resultant peptide-centric ‘altered self’ activates abacavir-specific T-cells, thereby driving polyclonal CD8<sup>+</sup> T cell activation and a systemic reaction manifesting as AHS.<sup>2</sup> We have also shown that carbamazepine, a widely used anti-epileptic drug associated with hypersensitivity reactions in *HLA-B*<sup>*</sup>15:02 individuals, binds to this allotype, producing alterations in the repertoire of presented self-peptides. These findings highlight the importance of HLA polymorphism in the evolution of pharmacogenomics as well as providing a general mechanism for some of the growing number of HLA-linked hypersensitivities that involve small molecule drugs. The potential of other drugs, environmental agents and metabolites on altering antigen presentation will be explored in projects that examine additional drugs and microbial metabolites.

**HLA Molecules behaving badly**

Human Leukocyte Antigen (HLA) B27 belongs to the MHC (major histocompatibility complex) class I family. These molecules are able to bind thousands of peptides and present these on the cell surface for recognition by T lymphocytes. The array of peptides bound by these molecules is derived from virtually every self or pathogen-encoded protein within the cell.

HLA B27 is strongly associated with several inflammatory rheumatic autoimmune diseases including ankylosing spondylitis (AS). Although other genetic associations have recently been identified in these disorders, none are as strongly linked to spondyloarthritis as the HLA B27 locus. Indeed, the association of HLA B27 with AS is the strongest association of any HLA gene with a clinical disorder. Approximately 90% of all AS patients express the HLA B27 genotype.

Interestingly, onset of AS in HLA B27<sup>+</sup> individuals often follows gastrointestinal infection with intracellular gram-negative bacteria (e.g. *Salmonella*) implicating infection as the trigger for induction of autoimmunity. Several theories have emerged trying to explain the role of HLA B27 and bacterial infection in AS pathogenesis. One theory is based on the observation that HLA B27 has a propensity to form homodimers at the cell surface. These unusual HLA B27 forms themselves or their peptide cargo might serve as target for immune receptors and trigger aberrant immune responses. In addition, their formation may be accelerated during bacterial infection.

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Molecular model of a HLA B27 homodimer (adapted from McMichael and Bowness Arthritis Res 2002 4:S153)


Rossjohn Laboratory – Infection and Immunity

The academic research program within this laboratory is focused on understanding the processes that control infection and immunity, specifically host recognition, responses developed by the pathogen. The human population is constantly under threat of microbial attack. The survival of our species reflects a delicate balance between infection and immunity. Whether an individual mounts an effective immune response or succumbs to microbial infection is critically dependent on host proteins interacting effectively with microbial antigens, versus microbes developing sophisticated strategies of invasion and immune evasion.

Anti-viral immunity; T-cell mediated organ transplant rejection; T-cell development; celiac disease; autoimmunity; HLA-linked drug hypersensitivities; Natural Killer cells, Natural Killer T-cells; lipid-mediated immunity - these are critical aspects of the immune system that our laboratory is investigating (see Borg et al., *Nature* 2007; Pang et al., *Nature* 2010; Vivian et al., *Nature* 2011; Illing et al., *Nature* 2012).

The flip-side to immunity, infection, represents the second main thrust of our research program. Bacteria have developed a suite of strategies to overcome the defenses of the immune system. Components that assist in immune evasion, and essential cellular machinery that is unique to a given microbe are investigated (Paton et al., *Nature* 2006; Byres et al., *Nature* 2008).

Our research program uses numerous biochemical and biophysical techniques including protein expression and purification, surface plasmon resonance and three-dimensional structure determination with the use of the Australian Synchrotron. Further, cellular immunology techniques are taught within the laboratories of the collaborators of the Rossjohn laboratory. The laboratory is funded by the National Health & Medical Research Council (NHMRC), the Australian Research Council (ARC), Anti-Cancer Council, National Institutes of Health, Roche Organ Transplantation Fund, Association for International Cancer Research and is an integral part of the ARC Centre of Excellence in Structural and Functional Microbial Genomics.

Researchers from this laboratory have been awarded various fellowships/honours including the Premier’s award, NHMRC Dora Lush Postgraduate research scholarships, NHMRC CJ Martin Fellowships, NHMRC Peter Doherty Fellowships, NHMRC CDA fellowships, EMBO fellowships, and ARC QEII fellowship, Victoria Fellowships and ARC Future Fellowships

Honours projects are available in the fields of adaptive and innate immunity.

Contact details:

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5. Molecular Cell Biology:

**Autophagy – eating your way out of trouble!**

**Professor Rod Devenish**  Dr Mark Prescott

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**Introduction**

All eukaryotic cells degrade (or turnover) parts of their internal structure including organelles by a process called autophagy (“self eating”) that occurs in a specialized compartment of cells - the vacuole (in yeast) or the lysosome (in mammals). In yeast, autophagy is mainly involved in cellular homeostasis (removal of damaged organelles) and adaptation to starvation, but in multicellular organisms (mammals) it is involved in a variety of additional processes such as programmed cell death and development of different tissue-specific functions.

Alterations in the levels of autophagy are linked to a growing number of pathological conditions including neurodegenerative diseases such as Parkinson’s, myopathies, some forms of cancer, and infection by pathogenic bacteria or viruses.

**Current work**

The turnover of mitochondria, the nucleus and other organelles by autophagy presumably serves as a means of quality control for organelle function. Mechanistically distinct forms of autophagy have been identified (see Figure 1). The molecular details and regulation of these processes and how they relate to organelle turnover are now becoming better understood, but we are a long way from having complete understanding. We are using fluorescent protein technology [Devenish et al. (2008) Methods in Enzymology, 451,109-131; Mijaljia et al. (2011) *J. of Visualised Experiments* (http://www.jove.com/details.php?id=27791]), together with other biochemical and molecular techniques, in yeast and mammalian cells to monitor turnover. This approach is providing new insights into the complex pathways and molecular mechanisms by which organelle autophagy occurs. We have established a ‘discovery pipeline’ to systematically screen a number of different yeast gene libraries and identify the molecular components and networks required for the regulation of mitophagy. For example, using our “Rosella” fluorescent biosensor in yeast (Figure 2) we have uncovered OTP1, a gene involved in an early phase of autophagy of mitochondria (mitophagy). Use of yeast as an experimental model first sparked the ‘explosion’ of knowledge concerning mammalian autophagic processes and continues to contribute new findings and understanding to the field.

**Autophagy as a host-cell response to bacterial infection.**

Successful microbial pathogens have evolved strategies to avoid or subvert autophagy thereby ensuring their survival within cells. Together with colleagues in the ARC Centre of Excellence in Structural and Functional Microbial Genomics we are looking at the molecular mechanisms by which the soil bacterium, *Burkholderia pseudomallei* achieves the avoidance or subversion of autophagy. In humans infection leads to Melioidosis, a disease endemic in tropical and subtropical areas. It is also a significant pathogen in many animals. This intracellular pathogen can escape from phagosomes into the host cytoplasm, where it replicates and infects adjacent cells. We are investigating the role played by autophagic processes in the intracellular life-cycle of *B. pseudomallei* in phagocytic cell lines, using confocal microscopy, intracellular survival assays and *in vivo* infection models. Our results [Gong et al. (2011) *PLoS ONE*, 6(3): e17852] show that an autophagy-related pathway, LC3-associated phagocytosis (LAP) provides a defence system for macrophage cells against invading *B. pseudomallei*. However LAP is relatively ineffective and most bacteria escape to the cytosol where they efficiently evade capture by canonical autophagy. Presumably various bacterial proteins act as effectors that interact with host cell trafficking factor(s) and contribute to modulation of host cell biology. We are seeking to identify such proteins and their functions during infection.

**Project Areas**

1. Autophagy of organelles, focusing on mitochondrial and nucleus turnover.
2. Autophagy in infectious disease; how autophagy can be avoided or subverted in microbial infection of mammalian cells.
3. Developing new biosensors for accelerating autophagy research.

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**Figure 1. Autophagy comes in different flavours.**

Mitochondria (right-hand side) are shown targeted for degradation in the mammalian lysosome.

**Figure 2. Example of Rosella assay output in yeast.** Here vacuolar red fluorescence indicates the delivery of mitochondria to the vacuole (white arrows). Under nutrient rich conditions (top panel A), mitophagy is not induced and no vacuolar red fluorescence is evident. Following induction of mitophagy in nitrogen-poor medium (bottom panel B) vacuolar red fluorescence indicates the occurrence of mitophagy.

**Figure 3. Confocal micrograph of RAW264.7 cells 6 h post infection with *Burkholderia pseudomallei* strain K96243 (magnification x600). Bacteria are stained red. Staining of filamentous actin (green) shows the presence of actin tails associated with infecting bacteria (red). The cell nucleus is stained blue.
Mitochondrial function and disease

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Introduction
Mitochondria have long been labelled as the “powerhouses” of eukaryotic cells as they house most of the machinery for production of ATP. However, recent advances in research make it clear that the functions of mitochondria are much more diverse. Defects in mitochondrial function are strongly correlated with many metabolic and neurodegenerative diseases. It is also therefore not surprising that many bacterial pathogens target mitochondria with toxin proteins during infection.

Mitochondrial dysfunction during infection
Many human pathogens that cause diseases ranging from gastrointestinal to neurological infections alter mitochondrial function. We are currently investigating the role of VacA from *Helicobacter pylori* (gastric ulcers and cancer) and PorB from pathogenic Neisserial species (meningitis and gonorrhoea). Both toxins are targeted to mitochondria. Despite the knowledge that these proteins (and many other toxins) localise at mitochondria, their modes of action remain enigmatic. VacA, which is made up of two sub-units, has effects on both the vacuole and mitochondria. During *in vitro* experiments VacA has been shown to form a pore when both fragments are added to a lipid bilayer. Our current research has confirmed that both subunits of VacA do indeed target to mitochondria and hence understanding what the two VacA fragments do at mitochondria is now a priority. The action of PorB from Neisseria is also only poorly understood.

![Mitochondrial dysfunction during infection](image)

Project areas

Assessing the effects of bacterial toxins on mitochondrial function.

1. Assessing the role of VacA on host cells with a focus on the mitochondria
2. Assessing the role of PorB at mitochondria.
3. Examining the functions of previously uncharacterised Neisserial toxins on host cells. We have recently completed proteomic analysis and discovered Neisserial proteins that are targeted to host cells via membrane vesicles. We are now in the process of elucidating their individual roles during infection.

Related techniques: mammalian tissue culturing, *in vitro* translocation assays, live and fixed cell fluorescence microscopy, biochemical assays, bacterial culturing.

Please refer to the laboratory web page for project updates and other projects may be offered upon enquiry.
Regulation of Nuclear Transport in Development and Viral Pathogenesis

Prof. David A. Jans

A. Viral pathogenesis: regulated nuclear trafficking of proteins from RNA viruses

RNA viruses are significant threats to human health, highlighted by recent fatal outbreaks of viruses such as swine flu and Hendra virus, and the continuing spread/persistence of viruses such as Dengue and HIV. Viruses often utilise and/or disrupt mechanisms of the infected host cell to ensure the precise subcellular localization of viral proteins, as well as mislocalize host proteins, with consequent increased virus replication and inhibited host anti-viral response. We are looking at a number of viruses of medical significance as models to study the importance of regulated subcellular localization of viral proteins in pathogenesis. The results can be used to identify new targets for the development of urgently needed anti-viral agents.

1) The NS5 gene product from the causative agent of dengue fever, dengue virus (DENV), localises predominantly in the nucleus, even though its function in virus replication is in the cytoplasm. We have shown that NS5’s signal dependent trafficking into and out of the nucleus is essential to virus replication. Hons. projects cosupervised by Drs. Kylie Wagstaff/Johanna Fraser are aimed at understanding the mechanisms of regulation of NS5 nuclear trafficking e.g. by phosphorylation, and examining interactions of NS5 with host-cell proteins both within the nucleus and cytoplasm and determining the effects of these associations on virus replication. Techniques: PCR, cloning, cellular transfections, viral infections, immunoprecipitations, RT-PCR, siRNA and confocal microscopy.

Fig 1. Anti-phosphorylation site mutant (PM) flag-tagged M protein but not wildtype M (WT) form cytoplasmic filaments along microtubules (MTs) and not actin within transfected cells. Both MWT and MPM form cytoplasmic filaments within transfected cells, but only MPM shows strong localisation (white arrows) to the MT network, implying that dephosphorylation at this site plays a key role in M-MT interaction.

2) Respiratory syncytial virus (RSV) is an RNA virus of significance that accounts for more human deaths than influenza each year. RSV matrix (M) protein plays an important role in pathogenesis through its ability to shuttle into and out of the nucleus. This shuttling is regulated by phosphorylation/interactions with the cytoskeleton (Fig. 1). In the cytoplasm M facilitates virus assembly, whereas M’s role in the nucleus is to inhibit host transcription, thus shutting down the host cell immune response. Employing a number of proteomic approaches (yeast 2-hybrid, mass spectrometry), we have identified a series of potential M-protein interaction partners that may aid M in its nuclear and cytoplasmic function(s). Hons. projects cosupervised by Dr. Leon Caly are focused on determining the mechanism of inhibition of transcription by M, validating newly identified nuclear and cytoplasmic targets of M and the effect of knocking down (siRNA) these targets on RSV infection and M subcellular localisation/trafficking.

3) Rift Valley Fever Virus is a serious emerging pathogen affecting humans and livestock in sub-saharan Africa, where recurrent epidemics have killed hundreds of thousands of animals, several thousand humans and caused significant economic losses. The non-structural proteins (NSs) is thought to be the main virulence factor, whereby loss of the protein renders the virus infectious, but not pathogenic. NSs localizes to the nucleus of the infected cell where it forms unique ribbon-like structures (Fig 2), and plays a role in subverting the host Interferon response. Despite being critical for viral infectivity, very little is known about the nuclear import mechanism of NSs. This project will investigate the nuclear localization signals and transport pathways responsible for NSs nuclear localization, with the AIM to identify a novel target for the development of anti-viral therapeutics.
Fig 2. Flag-tagged NSs protein from Rift Valley Fever Virus. NSs forms filamentous structures in the nucleus that do not contain chromatin. Left panel- anti-FLAG antibody, Middle panel- DAPI stain, Right Panel- Merged image.

4) Many viruses depend on nucleocytoplasmic trafficking for infection, viral protein nuclear import is an attractive target for therapeutic intervention. Hons. projects cosupervised by Dr. Kylie Wagstaff will screen for and characterise novel inhibitors that specifically block the interaction of viral proteins with their respective nuclear transporters, for their ability to inhibit viral replication (Fig. 3).

Fig. 3. Nuclear transport inhibitors can block Dengue virus replication. A short pretreatment with the inhibitor that blocks NS5 nuclear import blocks Dengue virus production.

B. Negative regulators of nuclear import with roles in development

(cosupervised by Dr. Kylie Wagstaff)

Cell differentiation is controlled by changes in transcription within the nucleus, mediated by access to the nucleus of proteins such as transcription factors through cellular transporters called importins (IMPs), which accordingly are also central to the developmental process. IMP13 is an IMP implicated in developmental pathways in the testis/brain/foetal lung, and linked to X-linked mental retardation/childhood asthma. Uniquely, IMP13 appears to be able to mediate transport bidirectionally, with a novel testis-specific truncated variant appearing to function as a negative regulator of nuclear import (NRNI). Hons. projects will examine how a single IMP can mediate transport bidirectionally as well as act as inhibit nuclear import, and assess the range of proteins interacting by this novel IMP (the import-13-ome).

Techniques: confocal laser scanning microscopy, in vitro nuclear transport assays, protein-protein interaction assays, robotic screening, FACS analysis, virological/ immunochemical techniques, gene reporter assays, viral reverse genetics and yeast 2-hybrid systems.
Our lab’s major research area concerns the plasma membrane NADH-oxidoreductase (PMOR), electron transport across the plasma membrane and its involvement in iron import into mammalian cells. Iron is important for cellular survival: without iron, every cell will die. In its physiological form, extracellular iron is complexed by chelators, molecules that bind to metallic ions. The most important chelators are the protein transferrin and the metabolite citrate. In order for iron citrate to be taken up by a cell, iron is thought to first have to been reduced from iron (III) to iron (II). For this reduction to occur, the electrons must be supplied from inside the cell. Our interest lies in understanding how cells transport electrons across the plasma membrane and it was long suggested in the literature that the PMOR is involved in this reduction.

We have analysed the cellular uptake of iron from iron citrate in detail, and found that vitamin C (ascorbate) is needed to reduce iron and to take it up. Cells actively export vitamin C for this purpose and take up oxidised vitamin C (dehydroascorbic acid). The cells then recycle it back to vitamin C and release it for further iron reduction (Fig. 1). In this way, vitamin C from the cells supplies electrons for this reaction and the subsequent uptake of iron.

**Projects available**

**Characterisation of the ascorbate-stimulated plasma membrane ferricyanide reductase**

We have made progress in the characterization of the ascorbate-stimulated plasma membrane ferricyanide reductase(s). This project will look at members of the cytochrome b561, especially Dcytb, and characterise their enzymatic activities (see Lane et al. (2010) *Biochem. J.* 428, 191-200; Fig. 2).

**Techniques to be used:** Cell culture, ferricyanide reductase activity measurements, siRNA, potentially liposome formation, potentially some re-cloning.

**Characterisation of the role of RCAN1 in iron homeostasis and redox signalling**

**Supervisors Drs A Lawen and MA Pritchard**

RCAN1 is overexpressed in individuals with Down’s syndrome (DS). DS brains as well as brains from transgenic mice over-expressing RCAN1 show increased hippocampal iron accumulation. In cells, over-expressing RCAN1 to a similar level as in DS, we observed increased sensitivity to oxidative stress. We plan to conduct basic molecular and cellular analyses of the effects of over-expressing RCAN1 on neuronal iron accumulation and oxidative stress.

**Techniques to be used:** Cell culture, iron uptake measurements, total antioxidant capacity measurements, cellular redox potential measurements, cell death studies.

For further information go to:

Mechanisms of viral immune evasion and pathogenicity

RNA viruses represent significant threats to world health, highlighted by the continued reemergence of zoonotic pathogens such as rabies/lyssaviruses (which account for >55,000 human fatalities/year) and Ebola in human population, as well as recent fatal outbreaks of Nipah and Hendra viruses in human and animal populations. Central to the pathogenicity of these viruses is their ability to evade or disable the host’s antiviral immune response, which is mediated by interferon [IFN]. Using a combined approach including diverse technologies such as high-end cell imaging techniques, subcellular trafficking assays, cell signalling assays, molecular interaction analyses, proteomics, recombinant virus technology and in vivo pathogenesis models, we aim to understand the mechanisms underlying viral immune evasion. This work has uncovered key roles for the subcellular trafficking of specific virally encoded proteins (“IFN antagonists”) in subverting the IFN system by mechanisms dependent on their ability to localize variously in nucleus and cytoplasm, and to interact with the cellular cytoskeleton (Figure). Importantly, we recently found that this highly regulated trafficking of IFN-antagonists is a vital pathogenicity factor in vivo, identifying it as a novel potential target for therapies against many currently incurable pathogens.

Honours projects are aimed at dissecting the complex mechanisms by which the cytoplasmic, nuclear, subnuclear and cytoskeletal targeting activities of IFN-antagonists are regulated and affect immune responses in the context of virus infection, and to determine the potential of targeting these processes to combat lethal virus infections. This research includes collaborations with the $500 million AAHL facility in Geelong, which provides internationally unique facilities for the study of highly dangerous viruses, as well as with the CNRS and Institut Pasteur (Paris), Gifu and Hokaido Universities (Japan), and UCI (US).

Microtubule association of rabies virus P3 protein. (Left) Confocal laser scanning microscopy image showing colocalization (yellow) of P3 and microtubule cytoskeleton. (Right) Automated tracing of the P3-microtubule associated network in 3D cell images.
Regulation and control of the Complement system in immunity

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The complement system is vital in preventing disease caused by infections. It is also thought to be involved in a number of diseases where excess inflammation occurs.

Structure of the MASP-2 complement protease.
The domain structure of MASP-2 is shown below the structure of the protease. The two CCP domains are on the left of the structure shown and the catalytic domain is on the right of the structure. The CCP domains interact with substrate proteins such as the C4 complement protein.

We are studying both the classical and mannose-binding lectin (MBL) pathways of complement activation. These pathways involve the sequential activation of proteins by a cascade of proteases. A particular focus in the laboratory is the initiating proteases of the two pathways, C1s and MBL-associated serine protease-2 (MASP-2). We are studying these molecules using a combination of X-ray crystallography, enzyme kinetics and a variety of binding assays to understand the interactions of the proteases with their substrates and inhibitors. Students will experience all of the above techniques in the projects described below.

Projects:
1. Mapping the active site of MASP-2 and C1s using site-directed mutagenesis in conjunction with mapping of substrate specificity using a combinatorial peptide substrate library.
2. Analysis of the action of small chemical inhibitors of complement proteases.
Fluorescent proteins – the genetic code for light

Dr Mark Prescott and Professor Rod Devenish
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Fluorescent protein technology has without doubt revolutionised the way in which we carry out experiments in the life sciences, and accordingly was the subject of the 2008 Nobel Prize for Chemistry. Few areas of biochemical research remain untouched by the technology. Fluorescent proteins such as the green fluorescent protein (GFP) cloned from the jellyfish *Aequorea victoria*, have been engineered and developed in many different ways to produce an enormous palette of proteins with fluorescent properties (see picture above for a selection) useful for monitoring a vast range of events in living cells. The commonly used GFP is just one member of a protein super-family found in marine organisms. Although each member folds to forms the same 11-stranded β-barrel (see picture below) a variety of different chromophores (the light emitting component buried inside the barrel) are found to form spontaneously. The chemical structure of the chromophore together with the complex network of interactions between the chromophore and the surrounding amino acid side-chains (the protein matrix) determine the myriad range of optical properties.

Members of this protein family possess a constellation of fascinating and biotechnologically useful features. For example fluorescence emission of different proteins can with light be ‘switched’ from green to red, cyan to green, fluorescent to non-fluorescent, or vice versa, in a process that can be reversible or non-reversible. Such photoswitchable FPs are useful as ‘optical highlighters’ or optical data storage systems. They are the foundation of emerging super-resolution microscopy techniques that allow cellular features 10 times smaller than can be seen with conventional light microscopy to be visualised.

Our aim is twofold: (a) understand the complex and subtle relationship between FP structure and optical properties, and (b) use newly acquired knowledge to design and engineer FPs for novel biotechnology applications. In particular we are exploring their use in the field of optogenetics. In this new and exciting field of research genetically engineered light-sensitive probes are used together with focussed light for switching processes ‘on’ and ‘off’ in living cells, tissues and intact organisms.

We are investigating several aspects of these colourful and fascinating proteins using a powerful interdisciplinary approach. In conjunction with Associate Professor Matt Wilce (Monash University) we are determining the X-ray crystal structure of a range of novel FPs. Some of the fluorescent protein crystals we have used to determine X-ray crystal structures are shown below. The photophysical aspects are studied in collaboration with A/Prof. Trevor Smith at the Ultrafast and Microspectroscopy laboratories (Melbourne University). In conjunction with Prof. Sean Smith (Centre for Computational Molecular Science, University of Queensland) we are using supercomputers to help model from first principles the optical behaviour of chromophores. This fundamental approach will help in the rational design of novel proteins with useful properties.

Project Areas
1. Engineering and characterization of FPs with useful optical properties.
2. Crystallization and X-ray structure of fluorescent proteins.
3. The development of FPs for use in optogenetics.
Membrane Biology Group
Dr Georg Ramm
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Our group studies the regulation of autophagy, an intracellular degradation process, and its role in diseases such as cancer. Our goal is to understand the spatial and temporal organisation of signalling events regulating autophagy. Autophagy is a multi-step intracellular membrane trafficking process that delivers cellular organelles and cytoplasmic structures to the lysosome for degradation. Autophagy is tightly linked to cellular metabolism and is increased during starvation. Our group uses a combination of electron microscopy, biochemical, molecular biology and fluorescence imaging techniques, with a focus on cutting edge imaging technologies such as correlated light and electron microscopy in the laboratory.

Illustration of correlative light and electron microscopy. A fluorescently-tagged LC3 protein, a marker for autophagy, is observed by life cell imaging in mouse fibroblasts (green, left picture). The same regions are sub-sequentially identified by transmission electron microscopy (middle panel, scale bar 5μm and right panel scale, bar 200nm). Image: Ben Padman.

Project 1: Regulation of the Protein Kinase ULK1 through subcellular localisation and protein-protein interaction: The Serine/Threonine Kinase ULK1 (Unc-51 like kinase) is emerging as the most upstream regulatory kinase in autophagy. Autophagy delivers cytoplasmic proteins and organelles to the lysosome for degradation and is upregulated during starvation to free up extra energy for survival, but is also needed to degrade malfunctioning organelles. With the induction of autophagy ULK1 is recruited from the cytosol to intracellular membranes and we aim to better understand this membrane recruitment process. It is likely that this recruitment process is regulated through protein-protein interaction and we have identified potential candidates through mass spectrometry. The aim is to further characterise these interactions and test the cellular function of these protein-protein interactions.

Project 2: Function and localisation of novel members of the RabGAP protein family: Molecular trafficking events are regulated by the Rab class of small GTPases. There are about 60 Rab GTPases known so far and they are distributed in a cellular organelle specific way. Their GTPase activity is regulated through more than 40 Rab GTPase activating proteins (RabGAPs). Of these only a few have been characterised. The function and distribution of the fast majority of RabGAPs is largely unknown at the moment. We aim to localise a number of novel RabGAPs and ask some fundamental questions about their function such as whether RabGAPs are distributed in an organelle specific way similar to their Rab targets. We will also investigate whether there is overlapping distributions/functions of RabGAPs or if there is unique specificity of each RabGAP for a particular membrane domain.
MOLECULAR BIOLOGY OF HUMAN FUNGAL PATHOGENS

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The research in my lab focuses on the major human fungal pathogen \textit{Candida albicans}. Fungal pathogens represent a substantial threat to human health. Fungal infections have become more prevalent in recent decades due to advances in medical treatments, and the mortality is very high, commonly 30-50%.

We work towards understanding the main virulence determinants of \textit{C. albicans}: formation of the cell surface (cell wall), the ability to form elongated cells called hyphae and formation of multicellular drug-resistant biofilms on surfaces. Our approach is interdisciplinary. We combine Genetics and Cell biology, Biochemistry, Systems biology (transcriptomics, proteomics), Bioinformatics approaches and Virulence studies, to understand how \textit{C. albicans} causes disease and why it is such a successful pathogen.

Project: Gene expression regulators of hyphal growth and biofilm formation
\textit{C. albicans} exists in three morphological forms, each with a role in virulence: (i) round yeast cells, for dissemination through the human body; (ii) elongated hyphae important for tissue penetration and (iii) biofilms-communities of cells forming on attachment to medical devices (e.g. catheters, pacemakers and prosthetic devices), which are extremely resistant to antifungal therapy.

We are screening for new regulators of these morphological transitions, focusing on:
(i) The co-regulators of transcription (such as the Mediator complex), which regulate the activity of RNA polymerase II;
(ii) RNA binding proteins that control mRNA stability and translation.

The projects in my laboratory will allow development of skills in microbial pathogenesis, molecular genetics, microscopy (confocal and electron microscopy), molecular biology and gene expression control (e.g. quantitative PCR, transcriptomics) and virulence studies in animal models.
6. Obesity & Diabetes:
Sleeman Lab
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Research Assistants: Victor Howard and Kristina Youngs
Honors student: Tara Jois

We are currently active in 2 main areas of Obesity & Diabetes Research

A. Ghrelin Biology: Ghrelin is produced primarily in endocrine cells located in the stomach, duodenum and is an endogenous regulator of energy homeostasis. It is unique in that it increases food intake (across a number of species) as well as decreasing energy expenditure and promotes adiposity in rodents. To further understand the function of ghrelin we have a number unique genetically modified mouse models at our disposal. These include mice deficient in ghrelin, its receptor GHSR or deficient in the ghrelin-activating enzyme, GOAT. We also have a number of in vitro projects looking at ghrelin function.

Ghrelin Project Areas:

1. Ghrelin and Insulin secretion: One not very well characterised function of ghrelin is its ability to inhibit insulin secretion from the pancreas. We believe that the protein tyrosine phosphatase, PTPRN2, is involved and want to characterize in a pancreatic islet cells line. This project will look at the effect of ghrelin on expression of PTPRN2 as well as on insulin secretion.

2. Ghrelin and microRNAs. MicroRNA’s are 20-25 nucleotide long non-coding RNAs that bind to, and usually down-regulate a number of specific target genes. We have found one microRNA, miR-153, to resides in a target gene of ghrelin. The major aim of this project is use an in vitro cell line to determine whether ghrelin is able to modulate expression of miR-153.

3. Ghrelin and the HPA axis: In collaboration with Dr Zane Andrews lab we are interested in the role that ghrelin may have in activating the Hypothalamic-Pituitary-Adrenal (HPA). The primary aim of this project in collaboration with Dr Andrews group is to determine why the ghrelin specific acyl-transferase (GOAT) is expressed in the pituitary.

B. Carbohydrate Response Element Binding Protein (ChREBP) Biology: An important transcriptional regulatory factor that may be involved in the development of fatty liver, insulin resistance and possibly Type 2 Diabetes is the carbohydrate response element binding protein (ChREBP). To understand its function and expression pattern we have generated mice than are deficient in ChREBP.

ChREBP Project Areas:

1. What is the physiological role of ChREBP? Mice that are deficient in ChREBP are breeding and await physiological characterization. This project will look at how these mice respond to various physiological challenges, such as special fat diets and fasting.

2. In what tissues and where is ChREBP expressed? The ChREBP deficient mice have a reporter gene (LacZ) put in place of ChREBP gene. By sampling, sectioning and staining of various tissues we can identify the expression patterns. The primary aim therefore is to characterize the cellular and tissue expression pattern of the reporter gene, and hence the ChREBP gene.

3. How does ChREBP work? The exact mechanism by which the ChREBP transcription factor works is not known at the present time. We have generated an in vitro cell system to study the phosphorylation and translocation of ChREBP.
To study any these questions, students will be trained in all the necessary techniques. For *in vitro* studies tissue culture techniques, DNA transfections, Western blotting, and quantitative real time polymerase chain reaction (qRT-PCR) will be used. The more physiological studies will involve some animal handling, physiological testing and tissue collection. Each of the projects above are ongoing projects in the lab but they could be expanded in any number of possible directions if the student desires. Students will be in a great lab environment, learn new cutting edge techniques and have the opportunity to help us understand the physiological basis of Obesity and Type 2 Diabetes.
7. Structural Biology:

**Peptidomimetic drug design ad membrane nanotechnology**

*Professor Mibel Aguilar*

*Email: mibel.aguilar@med.monash.edu.au*

Professor Aguilar has a major research program that includes the role of peptide-membrane interactions in a variety of disease states, β-amino acid-based peptidomimetics and the development of new analytical tools for isolating membrane proteins.

**Membrane-mediated biorecognition & membrane proteomics**

The interactions between peptides and lipids are of fundamental importance in the functioning of numerous membrane-mediated biochemical processes including antimicrobial peptide action, hormone-receptor interactions, drug bioavailability across the blood-brain barrier and viral fusion processes. Indeed, a major target of modern biotechnology is the design of new potent pharmaceutical agents whose biological action is dependent on the binding of peptides with lipid-bilayers.

*Model of the interaction of different peptides & proteins with the plasma membrane.*

We have developed novel procedures to study the conformation and dynamics of peptides during their binding and transport across the lipid bilayers of biological membranes. The future directions of this project area in collaboration with Professor Frances Separovic (University of Melbourne) focuses on exploiting these new surfaces in characterising the molecular basis of antimicrobial peptide action and the direct design of more potent and selective antimicrobial peptides as leads from new therapeutics for the treatment of bacterial infections.

In collaboration with Professor Wally Thomas (University of Queensland) we are also exploring the role of membranes in the study of membrane-mediated receptor activation using the G-protein coupled angiotensin receptor since it has been shown that receptor expression, affinity, signaling and trafficking is crucially dependent upon the membrane interactions.

*Model of the angiotensin receptor and its interactions with the cell membrane*

We are also applying these techniques, in collaboration with Prof David Small (University of Tasmania), to studying the role of membrane-binding in the cellular toxicity associated with amyloid formation in Alzheimer’s disease and other neurodegenerative diseases.
Therapeutic Peptides in Cardiovascular Disease

The action of most neuropeptides is terminated by specific extracellular peptidases and these enzymes therefore play an important role in the regulation of the function of the central nervous system. The availability of inhibitors of these enzymes is important for characterising the role of these enzymes in peptide signaling in the brain and ultimately for the development of new therapeutic agents for the treatment of cardiovascular disease. In collaboration with Prof Patrick Perlmutter (School of Chemistry), Prof Rob Widdop (Dept of Pharmacology) and Prof lan Smith we are focussing on a number of enzymes and receptors.

Membrane-bound aminopeptidase P (AP-P) also participates in the degradation of bradykinin in several vascular beds. Together with angiotensin-converting enzyme, AP-P is responsible for a large proportion of the breakdown of bradykinin. Since bradykinin exhibits potent vasodilatory and cardioprotective effects, there is a therapeutic benefit to inhibiting these enzymes and increasing endogenous levels of bradykinin.

ACE2 is a very recently discovered enzyme and is expressed largely in the kidney and heart suggesting important functions in cardiovascular and renal systems and currently there is an enormous interest in this enzyme as it has been proposed to be an essential regulator of heart function in vivo.

We are currently designing novel peptide and peptidomimetic based inhibitors of these enzymes in order to develop more effective approaches for the treatment of cardiovascular disorders.

![Image](image.png)

*The proposed mechanism of action of ECE, 24.15 & 24.16. These sites are targets for new drugs.*

New Nanostructured Functional Materials

Supramolecular self-assembly is a rapidly emerging approach for the design of new materials for the increasingly exacting requirements of biomedicine and engineering applications [refs]. Recently, significant progress has been achieved in the design and synthesis of nano-to-micro scale self-assembled fibers. There is, however, a critical need to bridge the nano-to-visible scale and establish a universal strategy for fiber production from the nano to millimeter or even meter sizes using small molecule self-assembly.

Together with Prof PAtreik Perlmutter and Dr Adam Mechler (LaTrobe University), we have discovered that β1-peptides self-assemble to form a variety of new materials. Identification of the underlying mechanism of formation of these new materials is currently underway. The ultimate aim of this program is the ability to design and manufacture high performance materials with designated functions.

![Image](image.png)

*A single molecule of Abeta imaged by Atomic Force Microscopy (AFM)*

Project Areas


3. Design of novel nanomaterials for biomedicine and nanotechnology

Introduction
Pathogenic bacteria and parasites have an arsenal of surface and secreted proteins to allow them to conquer the many unique niches they occupy throughout the course of infection. These proteins have varied functions such as adhesins for attachment to cells, toxins and virulence factors that manipulate or corrupt both host immune responses and cellular functions.

We use a combination of biochemistry, biophysical and X-ray crystallography approaches to determine the molecular role of these proteins in bacterial pathogenesis. This will not only unravel key aspects of microbial pathology, but will also provide a range of novel antimicrobial drug targets.

These studies will form basis of further studies to capitalize on the wealth of bacterial genomic data. The encoded proteins from such genomes provide an invaluable resource for the systematic examination of bacterial physiology, host-pathogen interactions and microbial pathogenesis.

Current work
AB5 toxins (in collaboration with Prof. James and Dr Adrienne Paton, University of Adelaide)
AB5 toxins are an important family of toxins that cause massive global morbidity and mortality, accounting for over 1-2 million deaths each year, particularly amongst children in developing countries. AB5 toxins exert their effects in a two-step process: (i) binding of the pentameric B subunit to specific glycan receptors on the target cell surface; (ii) internalisation of the AB5 toxin, followed by A subunit-mediated inhibition or corruption of essential host functions. The above AB5 toxins ultimately act on cytosolic targets, and so must be internalised, transported to the appropriate site and translocated across the respective organelle membrane. The AB5 toxins from each sub-family possess unique properties that arise from differing catalytic activities of the A subunit and/or differing receptor specificities of the B subunit.

Current projects
1) Understanding the structure and function of several novel AB5 toxins
2) Investigating the evolution of AB5 toxins


Malaria Translocon (in collaboration with Prof. Brendan Crabb (Burnet Institute) and A/Prof. Tania de Koning-Ward, (Deakin University).
Several hundred malaria parasite proteins are exported beyond an encasing vacuole and into the cytosol of the host erythrocyte, a process that is central to the virulence and viability of the causative Plasmodium species. The trafficking machinery responsible for this export is the newly discovered translocon of exported proteins (PTEX). The PTEX translocon complex is ATP-powered, and comprises heat shock protein 101 (HSP101; a ClpA/B-like ATPase from the AAA+ superfamily, of a type commonly associated with protein translocons), a novel protein termed PTEX150 and a known parasite protein, exported protein 2 (EXP2). EXP2 is the potential channel, as it is the membrane-associated component of the core PTEX complex. Two other proteins, a new protein PTEX88 and thioredoxin 2 (TRX2), were also identified as PTEX components.
**Current projects**

Determining the structure and function of the various components of malaria translocon


**Excreted/secreted (ES) proteins** (in collaboration with Prof. Robin Gasser University of Melbourne, Dr Peter Boag, Prof. Alex Loukas (James Cook University) and Prof. Els Meeusen)

Parasitic nematodes that infect the gastrointestinal tract of humans and animals are of major socioeconomic significance. These parasites secrete a range of proteins that play role in host-pathogen interface via establishing infection or manipulating the immune system however very little is known about how these proteins function. However they have been shown to be ideal candidates for making vaccines against parasitic nematodes and as treatment for autoimmune diseases such as Crohn’s disease

**Current projects**

Identifying and understanding various ES proteins from parasitic nematodes using biochemical, X-ray crystallography and surface plasmon resonance assays.
Protein Misfolding in Disease and Biotechnology
Prof. Steve Bottomley
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Our laboratory investigates the molecular basis of protein misfolding both in disease and biotechnology. Our ultimate aims are to develop therapeutics for a range of devastating diseases and to develop tools that aid in the production of recombinant proteins for use as drugs or as nanomaterials.

Over the next 50 years as the world’s population ages we will see a dramatic increase in the incidence of neurological disease. The majority of these diseases, including Alzheimer’s and Parkinson’s disease, occur when a specific protein fails to fold correctly. In the same manner the use of recombinant proteins as therapeutics or nanomaterials is hampered by the fact that almost 80% of protein produced in *E. coli* misfold and form inclusion bodies.

Our goal is to study the molecular basis of protein misfolding and develop strategies to prevent it. We apply a multi-disciplinary approach to define the molecular basis of protein misfolding, including the techniques of protein engineering, NMR and X-ray crystallography, biological spectroscopy, electron and atomic force microscopy and cell biology. All of our projects benefit greatly from their collaborative nature with other research groups within Monash and elsewhere.

**Project area 1. Polyglutamine repeat protein fibrillogenesis and its inhibition:** The polyglutamine family of proteins causes neurological disease through protein aggregation. Our previous work has shown that ataxin-3 must undergo a structural change to form fibrils. The aim of this project is to determine the mechanism of fibrillogenesis and identify key structural intermediates in the process. This will involve the use of biophysical techniques such as fluorescence and circular dichroism spectroscopy, electron and atomic force microscopy. Once we have identified and isolated the species formed during fibrillogenesis we will perform cellular experiments to examine the toxicity of these species.

An additional polyQ project we have begun is a drug screening program which is aimed at preventing ataxin-3 misfolding. This project will involve high-throughput screening of drug libraries for compounds which prevent or slow down ataxin-3 aggregation. Once hits are identified the interaction of these compounds with ataxin-3 will then be analysed using a variety of structural and biochemical approaches. The most suitable compounds will then be used in cellular and drosophila models of the disease.

**Project area 2. Serpin Misfolding and Disease:** The aim of this project is to investigate the conformational changes involved in the misfolding and polymerisation of antitrypsin, which results in emphysema and liver disease. Our previous data has shown that mutations cause antitrypsin to adopt an intermediate conformation with a high propensity to aggregate. The aim of the present study is to determine the conformation of this transient intermediate species using a range of biophysical techniques in combination with site-directed mutagenesis.

A second project will involve the development of a high-throughput screen for small molecular weight compounds that prevent serpin aggregation. This project will utilize both structural biology, in particular NMR, and biochemical techniques to screen libraries of compounds to identify potential therapeutic molecules. If successful the molecules will be used to probe cellular models of the disease.

**Project area 3 Protein misfolding during acute brain injury (in collaboration with Dr Andre Samson).** We have recently discovered a suite of rapid disulfide-driven protein misfolding events that occurs in the brain during acute injury. The extent of protein misfolding is such that the entire nucleocytoplasmic compartment of degenerating neurons remains as an aggregated ‘corpse’. These misfolded corpses represent an endpoint of the cell death process. These observations have broad implications - as acute brain injury is a risk factor for numerous diseases including Alzheimer’s disease, Parkinson’s Disease and Motoneuron Disease.

This project involves a variety of established *in vivo*, cell culture, *in vitro* and *in silico* techniques and will aim to identify the proteins that undergo injury-induced misfolding and determine which biochemical/cellular parameters influence the ability of proteins to undergo injury-induced misfolding.
Project area 4. Development of Recombinant Technologies to Prevent Misfolding: The ability to rapidly express and purify proteins has revolutionised molecular medicine and biotechnology. However, many of the techniques used in these processes are time-consuming and of low efficiency. We have begun a program aimed at developing new methods of gene cloning and protein production with the aim of automating these processes and minimising inclusion body formation. The project will involve the development of high-throughput protein purification strategies and its automation. During this project you will gain experience in molecular biology, protein expression and purification and high-throughput automation.
Structural Biology and Bioinformatics

Associate Professor Ashley Buckle
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Our research is aimed at understanding how the structure and dynamics of proteins dictates their function. Current themes include:

- We use molecular dynamics simulations to understand the role of conformational change xibility in protein function. This demands high performance supercomputing, such as the Sun Grid and Orchard - an 800-core Apple supercomputer cluster, the largest of its kind in the southern hemisphere. Recent projects include:
  - Dynamics requirements for activity of Dihydrodipicolinate synthase (DHDPS). In collaboration with Professor Juliet Gerrard and utilising BlueFern, the University of Canterbury IBM BlueGene Supercomputer.
  - Role of dynamics in the autoantigenicity of Glutamic Acid Decarboxylase (GAD65).

Although these projects do not require computer programming skills, basic computing competence is necessary. The project would be suitable for students wishing to perform novel bioinformatics and computer modelling/3D visualisation research using high-performance computing at Monash and VPAC.

A major axis of research focuses on the mechanism of action of proteases involved in human disease. Projects involve structure based inhibitor design using crystallography and computational techniques. Examples include:

- The design of inhibitors of human Kallikrein-4 (with Dr Jon Harris, Queensland University of Technology)
- Inhibitors of human aminopeptidases (with Professor Ian Smith and Professor Mibel Aguilar)
- Active and inactive proteases from the Scabies mite (with Professor David Kemp, QIMR).

We are using X-ray Crystallography, Small Angle X-ray Scattering (SAXS), Surface Plasmon Resonance Imaging (SPRI), Biophysics and Molecular Modelling/Simulation to understand the interaction between the Type 1 Diabetes autoantigen Glutamic Acid Decarboxylase (GAD65) and autoantibodies. In collaboration with A/Prof Merrill Rowley, Prof Malcolm Buckle (CNRS/Ecole Normale Superieure de Cachan, France), and Dr Daniel Christ (Garvan Institute and University of New South Wales).

Computational and bioinformatics tools for structural biology: in collaboration with Monash e-Research Centre, VeRSI and the Australian Synchrotron, we develop software tools for protein structure determination, and bioinformatics tools and databases for structural biology. Recent highlights include:

- TARDIS - the world’s first federated repository for raw X-ray diffraction data.
- Grid computing to speed up structure determination by X-ray crystallography.
- REFOLD: towards understanding why some proteins can be refolded and why others cannot.

For details see our group web pages: http://pxgrid.med.monash.edu.au/projects/
Structural Virology Laboratory
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The Structural Virology laboratory aims at understanding the assembly and replication of viruses by combining molecular virology and structural biology approaches.
Our research produces 3-D molecular models of viruses and viral proteins to provide functional insights and opportunities to design novel antiviral therapeutics.

**Project Areas**

1. Understanding the replication of Hendra virus
2. Structure-function of the capsids of giant viruses
3. Crystalline armours of viruses
4. Development of crystalline microparticles as vaccine for the developing world

**The replication of Hendra virus**
This project aims at determining the 3-D structure of proteins that play a key role in the replication of Hendra virus. It will improve our preparedness against emerging henipaviruses and help filling major knowledge gaps in our understanding of Hendra virus and more generally of Mononegavirales, a large class of RNA viruses regrouping many significant pathogens.

**The capsids of giant viruses**
The recent discovery of giant viruses larger and more complex than some bacteria has blurred the frontier between viruses and cellular life. Our current research focuses on the unique assembly pathway that these large DNA viruses, such as mimivirus or poxvirus, have evolved to form their infectious particles. This project investigates the role of highly-conserved capsid proteins in this assembly process that differs from well-characterized viral families of enveloped viruses such as HIV or flu.

**Crystalline armours of insect viruses**
Spheroids and polyhedra are unique viral infectious forms consisting of a crystalline armour packed with hundreds of virus particles. The crystalline armour protects the virus from environmental insults so that it remains viable for years in soil, analogous to bacterial spores. We use advanced structural biology approaches including X-ray microcrystallography at the best synchrotron facilities worldwide to determine the molecular architecture and function of these infectious crystals. The resulting 3-D models also provide a structural basis to engineer these crystalline microparticles as robust vaccines particularly suited for the developing world.
1) Molecular holepunchers: From immunity to bacterial disease
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 (Rosado et al., Science, 2007). 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 both immunity and disease.

A) Hole punching proteins used by the immune system:
In collaboration with Professor James Whisstock and Dr Ruby Law.
The human immune system uses two key MACPF pore forming toxins to kill pathogens: perforin and the Membrane Attack Complex (MAC). Both are used to form pores in target cells. In the case of perforin, virally infected host cells are targeted by perforin which forms large channels on the membrane surface. These channels deliver pro-apoptotic enzymes such as granzyme B resulting in cell and virus death. The MAC is activated by the complement pathway to lyse pathogens such as Gram negative bacteria. We are studying the ultrastructure of the pores using single particle electron microscopy as part of an international collaboration with Professor Helen Saibil (Birkbeck College, London).
Lab skills taught include:
Bioinformatics, molecular biology, protein chemistry, X-ray crystallography, biophysical techniques, cell lysis assays and electron microscopy.

B) Bacterial MACPF proteins: Are they virulence factors?
MACPF toxins are related to the CDC toxins used by Gram positive bacteria to cause disease. Bacterial genomics is starting to identify bacteria other than Gram positive bacteria that have MACPF/CDC toxins. Such bacteria include Chlamydia, Bacteroides and Fransicella species of bacteria. However we do not know what these proteins do: are they pore forming toxins or do they play a different role? As part of an international collaboration with Dr Jason Huntley (University of Toledo, USA), this project aims to identify the role of a MACPF protein in the lethal bacterium Fransicella tularensis. This research will help us understand the role of MACPF proteins in all bacteria.
Lab skills taught include:
Microbial genetics, bioinformatics, molecular biology, protein chemistry, X-ray crystallography, biophysical techniques and cell lysis assays.

2) Structural studies of vaccine candidate against fowl cholera
In collaboration with Prof Ben Adler, Department of Microbiology and Dr Tamas Hatfaludi
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.

Current vaccines against P. multocida consist either of bacterins, which provide only limited protection restricted to homologous serotypes, or attenuated strains, which can revert to virulence. Therefore there is a need for more effective vaccines to control diseases caused by P. multocida. As a step towards developing protective vaccines against fowl cholera, a genomics-based approach was applied to the identification of putative vaccine antigens. This approach resulted in the identification of an outer membrane lipoprotein, PlpE, which, when delivered as a urea-solubilised protein, stimulated protective immunity against infection. This is the first report where a denatured recombinant protein, PlpE, whose role in immunity remains unknown, has provided protection against fowl cholera. This project will try to elucidate the mechanism of function of this protein and its immunological importance using X-ray crystallography, molecular biology techniques and virulence studies in animal model.
Structural Biology of Novel Molecular Drug Targets

Dr. Sheena McGowan

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We are interested in characterizing novel molecular drug targets. The lab has a strong research focus in the design of novel anti-malarial drugs as well as other parasitic and bacterial diseases. Primarily we are a structural biology laboratory using techniques in X-ray crystallography, biochemistry and biophysics to analyse our proteins of interest. We use this mechanistic information to design inhibitors or analogues with potential applications in human medicine. Honours projects offered in 2013 include:

**Project Area 1: Characterizing Novel Anti-Malarial Drug Targets**

The complex life-cycle of malarial parasites involves a reproductive cycle within host erythrocytes which is the cause of the clinical symptoms of malaria. During this blood stage, the parasites digest hemoglobin, a process critical for parasite survival. The final stage of this process is reliant on the activity of three metalloaminopeptidase enzymes, M1, M17 and M18. The absolute requirement of parasites to use aminopeptidase activity to digest host haemoglobin makes this process an ideal target for chemotherapeutic intervention strategies and the three enzymes attractive targets for future antimalarials.

The three enzymes are thought to work in concert to facilitate protein turnover however the mechanism by which each protease modulates and controls hydrolysis remains to be elucidated. A detailed understanding of this mechanism of action and control will be vital if we are to design inhibitors that can mimic the natural substrate entry and block the active site of these enzymes. Using structural biology, together with enzymology, we aim to characterise and further develop lead compounds that inactivate the malaria parasite. Multiple project areas exist for 2013. For further reading, see McGowan et al., PNAS 2009, McGowan et al., PNAS, 2010, Sivaraman et al., McGowan. J Mol Biol., 2012

**Project Area 2: Structural and functional analysis of the Toxolysin 4 (TLN4) protease from Toxoplasma gondii.**

Toxoplasmosis is a parasitic disease caused by the protozoan parasite *Toxoplasma gondii*. The disease rarely causes symptoms in healthy adults however in immunocompromised patients and pregnant women the disease can be fatal and/or cause serious developmental defects in the fetus. In collaboration with Prof Vern Carruthers (University of Michigan, USA) we are performing structural and functional studies on the M16A metalloprotease from *Toxoplasma gondii*, also known as Toxolysin 4, has been shown to have a role in host cell invasion and parasite development.

**Project Area 3: Purification and characterisation of toxin regulators from Clostridium difficile.** (Joint project with A/Prof Dena Lyras, Department of Microbiology).

Pseudomembranous colitis is an infection of the large intestine with an overgrowth of *Clostridium difficile*. It is the major cause of hospital associated diarrhoea in the developed world and is estimated to cost the US health care system ~ $3.2 billion / year. The disease is caused by large toxins produced by the *C. difficile* bacteria. In collaboration with A/Prof Dena Lyras (Microbiology) we study a novel toxin regulator found within the *C. difficile* proteome with the aim to determine the mechanism of regulation of toxin production.

**Project Area 4: Purification and characterisation of the Chironex fleckeri Toxin 2 (CfTX2) from the Australian box jellyfish.**

The Australian "box jellyfish" or *Chironex fleckeri* is arguably the most venomous creature in the world. In collaboration with A/Prof Wayne Hodgson (Monash Venom Group) we are studying the structure and function of key lethal protein factors from the box jellyfish. This will aid with current treatments of envenomed patients as well as future pharmacology targets. Recently one of two toxin genes was cloned and protein produced. This project would involve the purification of the CfTX2 protein and its biochemical and physiological characterisation.
**Protein Production Unit**

**Dr Noelene Quinsey**  
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The work in this laboratory is directed at the high throughput production of recombinant proteins. Our research efforts are focused on developing methods that allow high throughput approaches, to solve many of the problems that are both time consuming and inhibitory to research projects.  

**Project:** Many of the recombinants proteins produced by the research laboratories are produced as fusion proteins with solubility tags attached. These solubility tags are often required for the expression and purification of the proteins of interest. However, they may hinder downstream experiments if they are not effectively removed. Thrombin is a common protease that is used to cleave the bonds between the solubility tag and the proteins of interest. Sources of thrombin are commercially available, however the amounts of material required, enzymatic stability and the purity of this material for large scale use prohibits many of its uses in a research laboratory situation. The aim of this project is to develop methods for the production and detection of a more stable protease that can be utilized in a biotechnology environment. The project will involve site saturated mutagenesis, high throughput protein solubility screening, high throughput protein purification and enzymatic activity of the resulting mutant proteins.

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**Chemokine-Receptor Interactions in Inflammation and Infection**

**Associate Professor Martin Stone**  
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Inflammation is the response of a tissue and its microvascular system to injury or infection. A hallmark of inflammation is the accumulation of leukocytes (white blood cells), which remove pathogens and necrotic tissue by phagocytosis and proteolytic degradation. However, excessive leukocyte recruitment or activity leads to the release of toxic substances and degradation of healthy tissue, i.e. **inflammatory disease**.  

Leukocyte recruitment in inflammation is controlled by the expression and secretion of small proteins called **chemokines** at the site of inflammation and by the subsequent interaction of those chemokines with **chemokine receptors** located on the surfaces of circulating leucocytes. A detailed understanding of chemokine-receptor interactions is required in order to rationally develop novel therapeutic agents against inflammatory diseases.

**The Structural Basis of Chemokine-Receptor Recognition**

Humans express about 50 chemokines and about 20 chemokine receptors on an array of different types of leukocytes. The intricate coordination of leukocyte trafficking (migration to the right tissues at the right times) is controlled, to a large extent, by the specific interactions between the chemokines and their receptors. For this reason, we have a major research effort towards understanding the structural basis of these specific interactions. This is particularly challenging because chemokine receptors are G protein-coupled receptors (integral membrane proteins), making them difficult to isolate or characterize in detail. Our studies utilize a wide variety of approaches including: NMR studies of chemokine structure; binding and activity studies of mutant and chimeric chemokines and receptors; and peptide models of the receptors.
We have developed a family of novel proteins (dubbed “CROSS” proteins) in which chemokine-binding elements of the receptors are displayed on the surface of a soluble protein scaffold. The CROSS proteins bind to chemokines with the correct specificity and therefore can be used to determine the detailed structural interactions giving rise to specific recognition. A major goal of our current research is to determine the structures of the chemokine-CROSS complexes.

HIV Infection via the Chemokine Receptors CCR5 and CXCR4
Human immunodeficiency virus (HIV), the causative agent of AIDS, infects human blood cells by interacting with the cell surface protein CD4 as well as either of two chemokine receptors (CCR5 and CXCR4). The mechanism by which HIV surface glycoprotein gp120 interacts with CD4 has been characterized structurally but the subsequent interactions with chemokine receptors are less well understood. Building on our soluble mimics of chemokine receptors, we have begun a new project aimed at characterizing the interactions of chemokine receptors with the gp120/CD4 complex. This project will reveal the structural rearrangements of gp120 that lead to fusion between the HIV and target cell membranes. By identifying critical elements of gp120, the project will guide the development of anti-HIV therapeutics and vaccines that cannot be readily overcome by mutation of the virus.

The Role of Tyrosine Sulfation in Chemokine Receptor Recognition by Chemokines and HIV
The interactions of chemokine receptors with both chemokines and HIV gp120 are enhanced by post-translational sulfation of critical tyrosine residues located in the extracellular amino-terminal regions of the receptors. However, detailed molecular studies of the influence of sulfation have been limited to date by the integral membrane nature of the receptors as well as the difficulty obtaining homogeneously sulfated proteins. Using our CROSS system, peptide models, and a combination of synthetic and enzymatic approaches, we are now investigating the role of sulfation in controlling the binding of chemokine receptors to chemokines and gp120. In addition to enhancing our understanding of these specific systems, our studies will provide fundamental insights into how Tyr sulfation can be used to regulate protein-protein recognition.
Projects in Professor James Whisstock’s Laboratory

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We use structural and molecular biology to investigate protein function and dysfunction in immunity and developmental biology. Support for the laboratory includes an ARC Federation Fellowship, NHMRC and ARC grants, an ARC Centre of Excellence in Structural and Functional Microbial genomics together with funding from the Wellcome Trust and the Trans Tasman Commercialization Fund. Five honours places are available in 2012. Generally projects involve any or all of the following techniques: X-ray crystallography, bioinformatics, molecular and cell biology, enzymology and protein chemistry. Project areas include:

1) Perforin-like proteins in immunity and cancer (with Dr Michelle Dunstone [Biochemistry / Microbiology], A/Prof Phil Bird [Biochemistry] and Prof Joe Trapani [Peter MacCallum Cancer institute])

Membrane Attack Complex / Perforin-like (MACPF) proteins comprise a large superfAMILY of pore forming proteins that in mammals play key roles in immunity, cancer, and development. Perforin itself functions by forming pores in target cells that permit entry of cytotoxic proteases (granzymes). Target cell death then eventuates through granzyme-mediated apoptosis. Our group has recently determined the X-ray structure of the first MACPF protein as well as the structure of perforin (Rosado et al., Science, 2007 and Law et al., Nature 2010); these data revealed the surprising finding that MACPF proteins are distantly related to a lethal family of bacterial pore forming proteins, the cholesterol dependent cytolsins. We furthermore show that perforin functions in the opposite orientation to the CDCs. This project aims to build on this discovery and further characterize the molecular mechanism of MACPF pore formation with a view to developing small molecule therapeutics to control unwanted perforin function in immune driven diseases and transplant rejection.

2) Pore forming proteins in neural development, autistic spectrum disorders and cancer (With Dr Coral Warr [School of Biological Sciences] and Prof Phil Bird [Biochemistry]).

A large number of novel MAPCF proteins perform important, but poorly characterized roles in as embryonic development, neural development and cancer. For example, knockout studies on astrotactin have revealed this molecule is important for mammalian brain development and polymorphisms within the human gene encoding astrotactin-2 are strongly associated with autism spectrum disorders. The model organism Drosophila contains a single MACPF protein Tsl, which is essential for embryonic patterning. Together with biochemical and structural approaches, we are investigating the role of Tsl in later stages of development, for example in CNS development and in adult fly immunity. Further, in collaboration with Prof Phil Bird, we are studying the role of mammalian MACPF proteins such as DBCCR1 in normal cell function and in cancer.

3) Understanding how blood clots are dissolved (Dr Ruby Law, A/Prof Paul Coughlin and Prof James Whisstock)

The ability to properly control blood clotting is fundamental for life; defects in the clotting machinery can result in serious bleeding disorders, however, the failure to effectively break down clots (fibrinolysis) may result in diseases such as thrombosis. This project focuses upon the fibrinolytic protease plasmin, its precursor molecule plasminogen and the plasmin inhibitor antiplasmin. Plasminogen and plasminogen activators are key drug targets in cancer and clotting-related diseases. We have recently determined the X-ray crystal structure of plasminogen (Law et al., 2012, Cell Reports). This project aims to build on this foundation and understand how plasminogen is activated to form plasmin. The eventual goal of the work is to develop molecules that modulate plasmin function in the context of clotting disease and cancer.

4) Development of novel anti-anxiety drugs (Dr Ruby Law, Mr Chris Langendorf and Prof James Whisstock)

Perturbations in the levels of the essential neurotransmitter gamma-Aminobutyric acid (GABA) are linked to serious neurological disorders such as depression, post traumatic stress disorder, anxiety and schizophrenia. GABA production is mediated by two...
isoforms (65kDa and 67kDa) of Glutamic Acid Decarboxylase (GAD65 and GAD67). We have determined the structure of GAD how shown how this essential enzyme system is regulated. We now aim to identify and develop molecules that have the capacity to alter GAD enzymatic function with a view to treating anxiety and related disorders.

5) Understanding the structural basis for bacterial conjugation. (Dr Daouda Traore, Prof Julian Rood, Prof James Whisstock)

Bacterial conjugation is essential to the acquisition of virulence and antibiotic resistance genes and the evolution of “superbugs” that are resistant to most commonly available antibiotics. Using a combination of structural biology, biochemistry and microbial genomics we are starting to elucidate the molecular mechanism through which DNA exchange between Gram positive bacteria is achieved (Porter et al., *Mol Micro*, 2012)

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### Projects in the Wilce labs

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**Protein-RNA interactions in antiviral cellular defence and inflammation**

Protein-RNA interactions are integral to cellular biology – both in normal cellular function and also in cells subject to the stresses of viral invasion. Proteins are responsible for the detection of viral RNA, and initiation of the innate immune response. Proteins direct post-transcriptional regulation of cytokines produced as a result of cellular stress, and are responsible for preventing their over-expression. In some cases, cellular proteins that normally function in translational control are hijacked by viral RNA as part of the viral mechanism of replication in the cell. Underlying each of these types molecular events are intricate and specialised molecular interactions. Their understanding would greatly advance our knowledge of antiviral cellular defence and potentially lead to new means to combat virus-related disease and inflammatory disorders.

Our lab has specialised in the study of protein-RNA interactions, using biophysical and structural tools to better understand the basis for their affinity, specificity and conformational consequences underlying their mechanism of action. This proposal is to delineate specific protein-RNA systems relevant to antiviral cellular defence and including:

1) **The RIG-I family of viral RNA sensors.** This protein family, which includes RIG-I, MDA-5 and LGP2 help control viral infection and are responsible for the cytosolic detection of foreign dsRNA. Complex formation with viral RNA initiates a conformational change that stimulates downstream expression of interferons and other inflammatory mediators. This is a desired response in the case in antiviral defence, but unwanted in the therapeutic application of siRNA. We are currently characterising this molecular system to better understand the basis of RIG-I protein activation. This will facilitate the development of drugs that either augment the innate immune response against disease or repress it for the application therapeutic siRNA.

2) **The interplay of AU-rich element binding proteins that dictate the translation of cytokines.** Inflammatory mediators that are stimulated upon cellular infection are predominantly regulated at the level of mRNA. Many of their transcripts contain an AU-rich element in their 3’-UTR that is recognised by proteins including TIA-1, TIAR, AUF-1, TTP and HuR. Depending on which protein is bound to the mRNA, the transcript is either transcriptionally activated, repressed or undergoes decay. We are investigating that way in which these proteins interact with their target sequences. This will provide important insight into the regulation of inflammatory mediators.

3) **The role of PCBP in picornavirus replication.** Poly-C-binding proteins (PCBPs) are RNA binding proteins that normally play a role in the regulation of translation of mRNA containing C-rich elements. In a fascinating example of viral use of cellular proteins, however, picornavirus RNA recruits PCBPs to stabilise its internal ribosome entry site (IRES) to initial translation of the viral genome. We are characterising this interaction to reveal the principles of picornaviral replication and potential novel means of therapeutic intervention.
Targeting the Grb7 protein involved in cancer progression

Growth factor receptor bound protein-7 (Grb7) is an adapter protein, aberrantly overexpressed in several cancer cell types, that mediates the coupling of tyrosine kinases with their downstream signalling pathways via its SH2 domain. In particular, Grb7 signals the activation of the erbB-2 receptor, which plays a key role in the progression of poor prognostic breast cancers. Grb7 is over-expressed with erbB2 in a subset of human breast cancer cell lines and breast tumours, suggesting erbB2 signaling via Grb7 may be increased in these cancers. Grb7 also mediates signalling pathways from focal adhesion kinase (FAK) in the regulation of cell migration, also implicated in tumor progression. It is thus a prime target for the investigation of the potential of novel anti-cancer therapies. We are currently examining Grb7-SH2-specific cyclic peptides developed using phage display libraries using biophysical techniques. Structural and affinity measurements for Grb7-SH2 domain, as well as computational approaches are being used to develop more potent and specific molecules. Cellular studies of cell-permeable forms of these peptides will allow us to better understand the downstream effects of Grb7 and to serve as a starting point in the design of therapeutics to targeting Grb7.

New antibiotics against Methicillin Resistant Staphylococcus aureus (MRSA).

It is accepted that human health is challenged by the increasing problems of multidrug resistant bacteria and that the development pipeline for new therapeutics for the treatment of such bacteria is very lean. The most problematic hospital and community wide bacteria which have developed considerable drug resistance have been collectively named the ESKAPE pathogens and includes Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter spp. For example, S. aureus was observed to develop antibiotic resistance very soon after the introduction of penicillin and later to methicillin. By 2003 over 50% of S. aureus isolates found in US hospitals were methicillin-resistant S. aureus (MRSA).
New pharmaceuticals desperately needed. We have developed a anti-MRSA drug design research stream. It includes collaboration between a range of disciplines including: microbiology, biochemistry, structural biology, medicinal chemistry and computational drug design. Our key target is the essential enzyme, biotin protein ligase (BPL). We are targeting BPL from each of the ESKAPE pathogens. Currently, we have a number of lead drugs against BPL from S. aureus (SaBPL) which are undergoing testing in animals.

Recent publications
Hormones produced by the anterior pituitary called gonadotrophins (follicle stimulating hormone, FSH and luteinising hormone, LH), play important roles in regulating the growth of ovarian follicles and the release of oocytes from the follicles at ovulation. Despite this knowledge, little is known about the factors and mechanisms operating within the ovary that determine which follicles will be recruited into the growth phase, or selects those follicles which will ovulate and those which will die. A better understanding of how the ovary is regulated would allow us to address issues related to fertility, contraception and cancer.

1. **Role of ERβ in ovarian function (with Prof Peter Fuller)**

Estrogens are known to be important for fertility; their precise role and point of action in the development of ovarian follicles is still being elucidated. One of the important questions that remains is what actions do the individual receptors mediate. Our lab is particularly interested in the role of ERβ in the ovary. We have a number of animal models and techniques to address these questions. Isolated ovaries and ovarian cells will be subject to gene expression studies, including real-time PCR and microarrays, cell culture, histological studies including laser capture microscopy (LCM), and stereological analyses.

2. **NFκB signalling in the ovary (with Prof Peter Fuller and Dr Simon Chu)**

Nuclear factor-κB (NF-κB) designates a family of transcription factors that have been shown to modulate antiviral, inflammatory and immune responses. Our studies in ovarian granulosa cell tumour cell lines (COV434 and KGN) indicate that NF-κB signalling is constitutively activated. Despite this observation a role for NFκB signalling in folliculogenesis has not been elucidated. These studies will utilise our colony of of IKKβ conditional knockout mice to investigate the importance of the NFκB signalling pathway in ovarian function. These mice cannot activate NFκB signalling in the ovary. Histological analyses of ovaries at different stages of development, serum hormone analyses, gene expression studies and various physiological challenges will be undertaken to characterise the ovarian phenotype.

3. **Regulation of primordial and primary follicle growth**

We will establish a model system for investigating the earliest stages of folliculogenesis ie, the recruitment of primordial follicles into the growth phase and their transition into primary follicles. An *in vitro* based model utilising ovarian slices would allow us to manipulate the environment either by the addition of hormones and/or growth factors. Gene expression studies, histological and stereological analyses will form the basis of projects in this area.

4. **Role of TGF-β superfamily members in folliculogenesis**

TGF-β superfamily members have been shown to influence steroid and inhibin production and the proliferation and differentiation of cells within the ovary. Little however, is known about the capacity of specific follicle populations to transmit these signals that influence biological function. We will use established cell culture models and develop new follicle based culture systems, employ immunohistochemistry, real-time PCR, microarrays, in situ hybridisation and hormone assays to investigate TGF-β superfamily signalling pathways in follicle populations.
5. Is there a role for the mineralocorticoid receptor in ovarian function (with Prof Peter Fuller)
We are in the process of generating a granulosa cell-specific conditional mineralocorticoid receptor (MR) knockout mouse model. The ovary is a novel tissue to be investigating MR action since its primary function is in salt and water balance. Progesterone (in addition to aldosterone and cortisol) produced by the ovary may activate MR signalling with consequences for ovarian function. Histological analyses of ovaries at different stages of development, serum hormone analyses, gene expression studies and various physiological challenges will be undertaken to characterise the ovarian phenotype of these mice.

OVARIAN CANCER: Granulosa Cell Tumours

Professor Peter Fuller and Dr. Simon Chu
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Granulosa Cell Tumours of the Ovary
Granulosa cell tumours of the ovary are a subset of malignant ovarian cancers which differ from the more common epithelial ovarian cancers and thus need targeted investigation if they are to have effective specific therapies. We have confirmed in our tumour panel a recent finding that all adult GCT contain a single specific point mutation in the FOXL2 gene. This defines the tumour but does not explain why same GCT recur and are ultimately fatal nor how GCT should be treated given their poor response to conventional chemotherapy. We use two human GCT-derived cell lines and the tumour panel to address these key questions.

The current focus of our work is on:
- **NFκB signalling in GCT:** the basis of the constitutive activity in the cell lines and its potential as a therapeutic target;
- **tyrosine kinase signalling:** exploration of novel tyrosine kinase inhibitors as therapeutic options;
- **microarray analysis:** gene expression profiles and mutation screening in the tumour panel;
- **nuclear receptors:** estrogen receptor β and PPARγ are prominent in the tumours and cell lines; their role as tumour suppressors and therapeutic targets;
- the role of **apoptotic pathways** in pathogenesis and therapy;
- the significance of the **FOXL2** mutation in GCT.
ALDOSTERONE AND THE MINERALOCORTICOID RECEPTOR
Supervisors: Professor Peter Fuller, Dr. Morag Young and Assoc. Prof. Tim Cole
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The adrenal steroid aldosterone is a key regulator of blood pressure. Excessive aldosterone levels cause hypertension and contribute to the pathogenesis of cardiac disease. Our laboratory has a program of work focused on various aspects of aldosterone action and the diseases that may arise from this action. This project examines the receptor for aldosterone, the mineralocorticoid receptor (MR), which acts as a transcription factor to regulate gene expression. The MR is unique amongst nuclear receptors in that it has two physiological ligands: aldosterone and cortisol.

Schematic of the MR showing the N-terminal (NTD), DNA-binding (DBD) and the ligand-binding (LBD) domains.

Current projects include:

1. **MR blockers**: The molecular basis of the interactions of antagonists such as spironolactone and eplerenone as well as new non-steroidal agents.

2. **Interdomain interactions**: The laboratory has identified a novel interaction between the N- and C-terminal domains of the receptor molecule which is induced by aldosterone but not cortisol. The molecular basis of both this ligand-discrimination and the functional significance of the interactions are being determined.

3. **Interacting proteins-coregulators**: Nuclear receptors associate with coregulatory proteins in order to modulate gene transcription and can have profound effects on receptor activity. We have identified novel MR coregulators that are tissue and/or ligand specific. A full understanding of the coregulator/MR interactions in different cell types will both expand our understanding of MR biological actions and facilitate the development of tissue selective drugs with reduced side effects.

4. **DNA-binding**: Nuclear receptor signalling is mediated through binding to DNA at specific “response elements” (the classical mechanism), by interacting with other transcription factors (teethering/transrepression) and through “non-genomic” pathways. To better define these pathways in the MR, we have created a transgenic mouse in which the MR has been mutated to preclude DNA binding. The phenotype of this mouse will be analysed for gene expression, physiology, cardiovascular responses and behaviour.

5. **Tissue specific functions of the MR**: Using transgenic mice our laboratory has identified novel MR actions in macrophages and cardiomyocytes. We will now investigate the specific role of the MR in endothelial cells in the vessel wall to understand the function of MR in cardiovascular disease and high blood pressure.

These studies use molecular biology, RT-PCR, microarray, tissue culture, transfection and reporter assays, confocal microscopy and transgenic mice.
Skeletal Muscle Research & Therapeutics Development
Lab Head: Dr. Paul Gregorevic
Baker IDI Heart & Diabetes Institute

Skeletal muscle accounts for almost half a person’s body mass, yet many people are surprised to learn that physical frailty caused by a loss of strength is the primary cause of death among a significant proportion of older citizens, and patients with chronic medical conditions. Moreover, even a moderate decline in muscle strength caused by advancing age, bed rest or inactive lifestyle can dramatically increase the incidence and severity complicating medical conditions. In our lab, the goal is to understand the cellular mechanisms that regulate muscle growth and wasting, so that we can develop new methods of preventing or treating muscle-related illness. The research places particular emphasis on employing recombinant viral vectors designed and manufactured “in-house” to selectively alter gene expression in mouse models, and analyses using a host of established and cutting-edge techniques spanning biological/biomedical science. This approach allows us to interrogate the cellular mechanisms controlling muscle adaptation in vivo with previously unattained precision.

Prominent research themes within the lab include:
1. Programs of protein signalling associated with muscle growth/wasting;
2. Changes in gene expression implicated in muscle adaptation;
3. Causes of pathology in models of neuromuscular disease, aging-, and cancer-related wasting;
4. Development of novel genetic and non-genetic therapies to prevent or reverse the loss of muscle function in disease.

Interested students are strongly encouraged to discuss possible projects that might fit within these main aims. Co-supervision between complementary supervisors/labs can also be considered.

Our major goal is to improve human health through what we learn. By embracing novel technologies in our research, and collaborating with leading laboratories world-wide, we always strive to be at the leading edge of this field and in the development of new therapeutic approaches. Come join an exciting and rapidly developing new research program

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1. The molecular genetics of human sex determination

Intersex disorders – ranging in severity from hypospadias to complete sex reversal – are surprisingly common with estimates as high as 40% of all live births. These disorders usually result in infertility, genital abnormalities, gender misassignment and long-term psychological trauma. The cause of these problems is most often the failure of the delicate network of gene regulation that is responsible for the proper development of testes or ovaries in the embryo. This project aims at understanding how the key sex-determining transcription factors, SRY, SF1 and SOX9, regulate target genes required for the development of testes, and how mutations in these genes lead to aberrant sexual development in humans. The project involves use of molecular and cell biology techniques and mouse models of DSD.

2. Identification of novel genes required for gonadal development

Our aim is to identify novel genes that underlie human disorders of sex development (DSD). In collaboration with Monash University, we are undertaking an ENU mutagenesis screen in mice at the Australian Phenomics Facility (APF), which is a National Facility based at the Australian National University (ANU) in Canberra. As a mutagen, ENU offers several advantages: (i) it is efficient and potent, inducing ~1 mutation per 0.5-1 megabase (Mb) of the genome (30 exonic mutations per pedigree); (ii) the mutations are essentially random, hence any gene can be mutated; (iii) it creates single base pair (point) changes, hence replicates the most common type of spontaneous mutation in the human genome; (iv) different pedigrees of mice can carry different point mutations within the same gene, thereby creating an allelic series, each able to reveal a different function in the protein the gene encodes. We are currently screening 50 pedigrees per year. Mouse embryos at embryonic days 13.5 and 18.5 are screened for abnormalities in testicular and ovarian development. We have identified several mutant strains affecting testis development which are currently under investigation. The causal mutations will be identified by backcrossing to the inbred mapping strain C3H.

We are also undertaking a sensitized ENU mutagenesis screen to identify genes involved in sex determination. We found that heterozygous Sox9 knockout mice are sensitized for XY sex reversal. While mice lacking one copy of the Sox9 gene do not show a gonadal phenotype, mice lacking one gene copy of each Sox9 and Fgfr2 display partial sex reversal (ovotestes).

3. New genes involved in disorders of sex development

Our project aims to identify new genetic factors involved in rare disorders of human gonadal development using Array Comparative Genomic Hybridization (CGH).

Since the discovery of SRY as a major player in human sex determination, few other genes have been identified as involved in human gonadal development, when sex is determined in the developing embryo (7 weeks gestation). Most of these genes have been identified using mouse models and/or by classical genetics studies of individuals with disorders of sex development (DSD). Such people are atypical in terms of anatomic, chromosomal, or gonadal sex e.g. XX males and XY females. Importantly, changes in known genes can only explain genetically about 20% of disorders of sex development.

The understanding of disorders of sex development (DSD) is greatly hampered by the complexity of the phenotypes, the rarity of the patients and the lack of an appropriate technique to analyse and compare such patients.

The development of high resolution CGH microarray is the next powerful tool needed to overcome these difficulties and will likely produce new information on human gonadal development. Array Comparative Genomic Hybridization (CGH) measures DNA copy number differences between a reference genome and a sample genome. Using whole-genome array CGH, we can measure copy number differences in DNA across entire genomes.

Given the complexity of phenotypes to be analysed by CGH, it is crucial to narrow down the patient numbers to be analysed to very carefully diagnosed patients. We are currently screening several XY female patients which harbour a normal SRY gene, for copy number differences across their entire genome.

4. Functional and structural studies of ATRX-AR interaction

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ATRX syndrome is a severe X-linked recessive developmental disorder affecting males who carry mutations in the ATRX gene. In addition to severe mental retardation, α-thalassemia, and skeletal abnormalities, 80% of ATR-X boys show genital abnormalities including small testis and ambiguous or even female external genitalia. ATRX is a very large SWI/SNF-like protein with chromatin remodelling activity in vitro, whose function is largely undefined, as is the reason why mutations in this ubiquitous factor result in such a highly specific syndrome. Mice lacking Atrx from their testicular Sertoli cells show spermatogenesis defects and notably a reduction in the expression of androgen-dependent genes (Bagheri-Fam et al., 2011). By chromatin immunoprecipitation we found that ATRX localises at Androgen Receptor target gene binding sites. Androgen Receptor is a nuclear receptor with a crucial role in the functional maturation of Sertoli cells as well as in spermatogenesis. For example, cooperative interaction between ATRX and AR was required for maximal transcription of Rho5, an essential spermatogenesis gene.
and AR-target. This project will investigate in detail the protein-protein interaction between ATRX and AR. We will map the interaction site/s between ATRX and AR, and purify the relevant domains using protein biochemistry techniques, towards structural studies aimed at understanding how ATRX regulates AR-target genes. Bagheri-Fam et al. (2011) Defective survival of proliferating Sertoli cells and androgen receptor function in a mouse model of the ATR-X syndrome. *Hum Mol Genet.* 20:2213-24.

5. The transcriptional role of SRY in brain function
Parkinson's Disease (PD) causes involuntary movement or tremors. Interestingly, PD occurs 1.5 times more often in men than in women. We think this could be due in part to the sex chromosome differences between males (XY) and females (XX). We have evidence that SRY, the "male only" gene on the Y chromosome responsible for the presence of testicles, also makes SRY protein in men's brains. When SRY is removed from rats' brains, the male rats develop movement problems reminiscent of those seen in PD which are reversed when SRY levels are restored. The movement problems in PD and in these rats occur due to a reduction of an enzyme called TH which is essential for dopamine production. We have discovered that SRY can regulate TH gene transcription in human dopamine-producing cells. This project aims to understand at the molecular level how SRY controls TH. Secondly SRY protein levels will be investigated in men with PD and in rat models of PD to test the hypothesis that lower TH levels are a consequence of lower SRY levels. This would implicate SRY as a contributing factor in increased susceptibility of men to PD.