Towards a new understanding of the reproductive system: from non-coding RNAs to disease

Our group focuses on the elucidation of regulatory mechanisms that control gene expression during embryonic development. One of the most amazing biological processes is the development of a fertilised egg into a complex organism. It involves the orchestration of cellular processes such as cell proliferation, migration, differentiation and apoptosis, which is controlled by a delicate network of gene regulation and interaction. Disturbance of this network caused by gene mutation or misexpression during development results in malformation and malfunction of organs, diseases such as cancer and often lethality. Therefore, each of these processes must involve a large number of regulatory mechanisms.

Until recently our work centred around the conventional dogma, which states that gene activity is controlled by transcription factor binding to proximal promoters and/or enhancers adjacent to genes. We are now extending these studies to include the fact that gene activity is also regulated post-transcriptionally by non-coding RNAs (ncRNAs), such as microRNAs. In addition to investigating the role of microRNAs during development, we have discovered a new class of ncRNAs, uaRNAs (3’UTR-associated non-coding RNAs) that display a highly regulated stage- and sex-specific expression pattern during embryogenesis.

Our research uses mouse as a model system and integrates molecular, developmental, and cancer biology to study mechanisms of gene regulation by transcription factors as well as ncRNAs during embryonic development, concentrating on sex determination and gonadal development but extending to other developmental systems such as chondrogenesis.

The aims of our research are to address the interactions of the following questions:

1. What are the regulatory mechanisms underlying the development of the reproductive system with emphasis on ovarian development?
2. What are the roles of ncRNAs, specifically uaRNAs and microRNAs, aj during the development of testes and ovaries, and b) in tumour formation?
3. How does testicular and ovarian cancer develop?

RESEARCH PROJECTS

• Characterising the role of miR-202 during embryonic development
• Identification and analysis of upstream regulators and downstream target genes of miR-202
• Functional characterisation of uaRNAs during embryonic development and possible implications in cancer
• Studying the cellular and molecular regulation of foetal ovary development

KEY PUBLICATIONS

Biochemistry of protein prenylation

Over the past 15 years, it has become increasingly clear that post-translational modification with isoprenoids is a widespread phenomenon, affecting up to 2 percent of proteins in eukaryotic cells. In all cases that have been studied, such a modification has been shown to be crucial for protein function by modulating protein-lipid or protein-protein interactions. Most of the prenylated proteins are RabGTPases that have key functions in signal-transduction pathways. Much of our attention is focused on the understanding of prenylation of RabGTPases – the largest group of prenylated proteins. RabGTPases are modified by Geranylgeranyltransferase I (GGTase) - a 100 kDa heterodimer that catalyses the transfer of two 20-carbon geranyleranyl groups from geranyleranyl pyrophosphate (GGPP) onto C-terminal cysteine Rab's C-terminus GTPases.

The remarkable feature of RabGGTase is its ability to interact with more than 70 different Rab proteins. At the same time, the enzyme is strictly specific for the Rab family and no unspecific activity could be detected. RabGGTase is composed of tightly associated alpha and beta subunits and belongs to the family of protein prenyltransferases together with squalene transferase (FTase) and geranyleranyl transferase (GGTase).

Our aim is to understand the molecular mechanisms underlying specificity of RabGGTase and the evolution of protein prenylation mechanisms. We use a combination of biophysical methods such as fluorescence spectroscopy and X-ray crystallography with methods of cell and chemical biology to obtain a complete mechanistic model of protein prenylation.

RESEARCH PROJECTS

- Proteome-wide analysis of protein prenylation and its variation in human disease
- Quantitative analysis of protein-protein interactions using a novel in vitro translation system
- Understanding of the mechanisms regulating protein prenylation machinery
- Identification of small molecules modulating prenylation and acylation of RabGTPases

KEY PUBLICATIONS


The endosome at atomic resolution: structural studies of the endosomal trafficking machinery

Our lab is focused on understanding the basic processes of intracellular membrane trafficking within the secretary and endocytic systems of the human cell. We do this using a multidisciplinary approach that integrates high-resolution structural characterisation of essential membrane trafficking machinery by X-ray crystallography with biochemical and cell biological experiments guided by these mechanistic details.

We concentrate primarily on the process of protein sorting within the dynamic-organelles known as endosomes, which are key sorting stations for regulated exo- and endocytoses or cell suicide receptors, signalling molecules and many other cellular components. The regulated trafficking of proteins and their ligands between membrane-bound endosomal compartments, the plasma membrane and other internal organelles is a fundamental process in human cells, and indeed in all eukaryotes. Defects in the endosomal membrane transport system are linked to many different human diseases, including a number of cancers, lysosomal storage disease and hypercholesterolemia, and it is also exploited by bacterial toxins and viral pathogens such as HIV to gain entry into the cell.

Membrane sorting between secretory and endocytic organelles is primarily controlled by small carrier vesicles and tubules that are layered on their cytoplasmic faces by specific protein machineries. The roles of these protein coats are threefold: (i) to select transmembrane and lipid cargo to be packaged into transport vesicles (ii) to dock the vesicle with the donor membrane, (iii) to control vesicle budding and scission and (iv) to specify the final destination of the transport intermediates. Using a multidisciplinary structural biology/biochemistry/cell biology approach, our goal is to reveal now these machineries assemble, how they are recruited to membranes and how they control vesicle trafficking at the molecular level. Current work focuses on the multi-subunit retromer protein complex with a central role in directed transport of endosomal cargo proteins, the sorting nexus (SNX) family of proteins involved in membrane remodeling, and a novel family of amnestic-related trafficking proteins.

RESEARCH PROJECTS

- Structure and function of the retromer protein complex
- Analyzing the interaction of retromer with cargo proteins and regulatory molecules
- Membrane remodeling by the SNX protein family
- Structural studies of PX-domain proteins and complexes with effector molecules
- Structure and function of amnestic-related proteins

KEY PUBLICATIONS


LAB MEMBERS

PhD Students: Chetanya Khurana, Zaki Trimbos, Marla Kubala
Our group studies mammalian intracellular signalling. We are especially interested in the function of Ras proteins. These small GTP binding proteins operate as molecular switches in signal transduction pathways and are present in a mutant, activated state in many human tumours. Understanding the basic biology of Ras has major implications for the development of novel anticancer therapeutics.

Specifically, we are investigating how the Ras membrane anchors cooperate with the G-domain and peptide sequences flanking the anchor to drive lateral segregation. Our work suggests new models are needed to explain how lipidated proteins interact with, and use, the plasma membrane to generate signaling platforms.

We remain interested in how confinement of signalling complexes onto a 2D surface in general, and in plasma membrane nanodomains in particular, regulates the kinetics and sensitivity of Raf/MEK/Erk signal output. Similarly, as we develop our spatial and proteomic maps of the plasma membrane, we can address how the composition and organisation of the membrane alters in response to specific growth factors. The integration of complex spatial, kinetic and biochemical data sets increasingly requires mathematical modelling to generate and test our novel hypotheses of nanodomain structure and function.

We also have a major interest in characterising the K-ras ER to plasma membrane trafficking pathway and studying the biology of Ras prenyl binding proteins such as PDE delta.

RESEARCH PROJECTS

- Molecular mapping of the proteins and lipids of plasma membrane nanodomains
- Electron microscopic visualisation and quantitative characterisation of surface nanodomains to build up a high-resolution 2D map of the nanodomains of the inner plasma membrane
- Investigating the dynamic regulation of nanodomain localization of Ras and Ras interacting proteins in response to physiological stimuli
- Characterising the mechanism(s) whereby K-ras is transported to the plasma membrane
- Mathematically modelling Ras signal transduction
- Monte Carlo modelling of plasma membrane nanodomain dynamics

KEY PUBLICATIONS


The 8-cells of the endocrine pancreas are the sole source of insulin in mammals. Death of the 8-cells, or their abnormal processing, trafficking and/or secretion of insulin, results in the disease commonly known as diabetes: This disease is one of Australia’s national health priority areas and represents the fastest-growing epidemic internationally. More than 230 million people worldwide currently live with the disease, but this number is expected to rise to 350 million within 20 years. In 2007, the world spent an estimated US$215-375 billion to care for diabetes and its complications.

In particular, type 1 diabetes is one of Australia’s fastest-growing chronic diseases, and represents a life-long autoimmune disease that usually begins in childhood and results in premature death through health complications. Type 1 diabetes cannot be prevented, and a cure remains to be found.

Our group’s research is focused on understanding the basic mechanisms related to 8-cell function and dysfunction from a structural cell biology perspective, so that we can precisely identify how and where defects in these steps occur. By necessity, this work has led us to develop or advance techniques for the improved preservation and imaging of pancreatic 8-cells in situ within pancreatic islets of Langerhans isolated from both mice and humans, so that we are positioned to reliably elucidate the basic cell biology and physiology of the 8-cell — and islet biology more generally — through comparative studies of 8-cell structure-function.

To complement our move toward an integrated or more holistic approach to understanding cells as examples of complex systems, we have undertaken a multi-scale multi-resolution approach whereby we have started reconstructing entire mammalian 8-cells in 3D at both high (adenylate) and intermediate (15-20nm) resolutions. These approaches underpin the VisibleCell project (www.visiblecell.com) coordinated between the IMB and the Australian Centre of Excellence in Bioinformatics (ACB) at The University of Queensland. Our group’s data will uniquely inform advanced in silico studies of 3D cell and molecular organisation in mammalian cells that are focused on developing the capacity to model and predict cellular differentiation during normal development, as well as the pathophysiology of chronic diseases like type 1 diabetes.

New Research Project: Correlative structure-function studies of cis- and trans-Golgi membrane traffic in mammalian cells

This project combines imaging by light and electron microscopy with additional techniques for studying protein function at the molecular level, to elucidate how changes in the 3D organisation of cellular machinery can lead to fundamental changes in the function and health of mammalian cells. Although this work includes detailed investigation of the ‘insulin factory’, it has the potential to modify established concepts on membrane traffic and protein secretion well beyond the field of diabetes.

KEY PUBLICATIONS


Structure-function studies of the endocrine pancreas – comparative studies of mouse and human pancreatic islet biology
The cell surface in health and disease

Our group is interested in the organisation, dynamics, and functions of the plasma membrane. The properties of the plasma membrane rely on the specialisation of the plasma membrane into microdomains of specific function. We have particularly focused our attention on caveolae, a specialised domain of the cell surface with a distinct structure. Caveolae have been implicated in regulation of cell growth and in maintaining the balance of lipids in the cell. In addition, caveolae and caveolins, the major proteins of caveolae, have been implicated in a number of disease states including tumour formation, atherosclerosis, and muscular dystrophy. To study caveolae function and, in particular, the link between lipid regulation and cancer, we are using caveola-null mice, cells lacking caveolins, and zebrafish embryos. These systems are also being used to study the role of caveolin in muscle and the molecular changes associated with muscular dystrophy. We have recently discovered a family of caveolin coat proteins that regulate caveola formation and function. An additional aim of our work is to understand the link between caveolae and lipid-filled organelles termed lipid droplets, which are major storage organelles involved in obesity. We have shown that caveolins are essential for the formation of lipid droplets during liver regeneration.

RESEARCH PROJECTS

- Characterisation of the structure and function of a new family of caveolar coat proteins
- Caveolae and obesity: dissecting the link between caveolae and lipid-filled organelles
- Caveolae and caveolin-3 in muscle: analysing the role of caveolin-3 and caveolin in muscle development and in muscular dystrophy
- Caveolins and caveolin-interacting proteins in zebrafish: using zebrafish as a model system to understand the role of caveolins during development and the effect of muscular dystrophy mutants of caveolin-3 on muscle structure and function
- Clathrin-independent endocytosis: characterising the structure and function of a novel endocytic pathway in mammalian cells and the zebrafish
- Caveola formation and structure: studying caveola biogenesis and caveola structure in health and disease using electron tomography and novel cell systems
- Caveola formation in vivo: characterising novel nanovesicles

KEY PUBLICATIONS


Our research group studies protein trafficking in human and animal cells with the aim of mapping the cellular organelles and molecules that function in the secretion and endocytosis of disease-related proteins. In this work we use a range of cellular, molecular and biochemical approaches. Trafficking is a highly-dynamic process and studies in this field have been greatly enhanced by the development of fluorescent probes and microscopic techniques for imaging in living cells. Live cell imaging, combined with other forms of microscopy, has thus become a major core technology for the research in our group.

In epithelial cells we are studying E-cadherin, an essential adhesion protein and a vital tumour suppressor. E-cadherin is trafficked to and from the cell surface to regulate cadherin-based cell-cell adhesion and cell polarity. A main goal of this work is to understand how E-cadherin trafficking functions in morphogenesis and cancer progression. As a model system we grow epithelial cells in mini-organ cultures where the effects of gene expression or gene silencing can be analysed.

Cells of the immune system secrete tightly orchestrated arrays of cytokines to control immune responses. In macrophages we are studying the secretion of pro-inflammatory cytokines that contribute to the onset and progression of chronic inflammatory diseases. Understanding how they are trafficked and secreted may lead to the development of new therapeutic strategies in inflammation. Gene expression arrays, live cell imaging, FACS and biochemical approaches are used to map out intracellular pathways for cytokine trafficking and secretion. Recent progress has shown that we can manipulate cytokine secretion in mice and current efforts are focused on using this approach in the treatment of arthritis and inflammation of the stomach and bowel. Based on recent findings, we are now also studying the pathways for phagocytosis or ingestion of different microbes by macrophages.

RESEARCH PROJECTS

- Imaging live cells to create 3D and 4D maps of trafficking pathways: fluorescence imaging and computer modelling
- E-cadherin trafficking in epithelia: morphogenesis and tumorigenesis in cyst cultures
- E-cadherin and growth factor signalling in cancer cells
- Secretion of inflammatory cytokines in macrophages
- Secretion of cytokines in mouse models of inflammatory disease
- Phagocytosis and trafficking of microbes

KEY PUBLICATIONS


Role of growth hormone and related cytokines in growth, cancer, diabetes and obesity

Adult height is determined by the actions of growth hormone (GH) during childhood and adolescence. In the adult, growth hormone is an important metabolic agent regulating body composition, opposing the actions of insulin. In old age, growth hormone status determines life span, at least in animal models. We study the means used by growth hormone to achieve these changes, using a variety of approaches directed to the growth hormone receptor, from high-resolution protein structures to genetically-engineered animals.

The growth hormone receptor determines the degree of the cell response to growth hormone, which we originally cloned collaboratively with Goedert. Through FRIT, BRIT, crystallography and targeted mutagenesis we have developed a new model of how the GH receptor is activated by GH, involving realignment of receptor subunits within a constitutive dimer. An extension of this model describes how a rearrangement of an extracellular b-loop of the growth hormone receptor selectively controls ERK activation without influencing STATS activation through the use of an alternate Src kinase.

By creating targeted knock-in mutations to signaling domains within the GH receptor cytoplasmic domain, we have shown that enhancement of postnatal somatic growth by GH is dependent on its ability to activate the transcription factor STAT5. Because these mice become strikingly obese after 6 months of age, we are currently investigating the role of STAT5a/b in control of lipid and carbohydrate metabolism, including insulin secretion and action.

Establishing the molecular basis for GH-dependent liver regeneration

Investigating the feasibility of using GH receptor antagonists to block breast and prostatic cancer

KEY PUBLICATIONS

Conway-Campbell, B.L., et al. (2008). The extracellular domain of the growth hormone receptor interacts with coactivator activator to promote cell proliferation. Molecular Endocrinology 22: 2190-2202.


Cells are the building blocks of our bodies. Interactions between different cells are important to shape our developing bodies, and a range of diseases occur when those interactions are disturbed, including cancer and inflammation. My laboratory studies one set of cell-to-cell interactions, those that occur when cells attach to one another. We focus on the cadherin family of cell-cell adhesion receptors. Those critically determine the ability of cells to recognise one another and organise into coherent tissues. The importance of these receptors is emphasised by the fact that loss of cadherin function promotes cancer progression in epithelial tissues (such as the breast and colon) – the commonest form of human cancers. By understanding the basic biological mechanisms of cadherin-mediated cell recognition we thus hope to provide vital insights into the basis of developmental patterning and common human diseases.

Currently we focus on understanding how cadherins cooperate with the actin cytoskeleton, long believed to be central to cadherin function. Our experience makes it increasingly clear that this cooperation involves a complex interplay between adhesion receptors and diverse distinct states of the cytoskeleton that are coordinated by a variety of signalling pathways at the cell membrane. In particular, our work demonstrates that cadherin function as an actin-activated cell signaling receptors that stimulate pathways to regulate the actin cytoskeleton, thereby influencing cell shape, adhesion, and cell-cell cohesion. Relevant signals include the Rho family GTPTases and Src family kinases. These affect a range of cytostatic regulators, including actin nucleators, cross-linking proteins, scaffold and the myosins I and VI.

KEY PUBLICATIONS


RESEARCH PROJECTS

• Regulation of the actin cytoskeleton by E-cadherin

• Cooperation between cadherins and myosin motors at cell-cell contacts

• Cooperativity between cadherins and microtubules

• Cadherin signaling to Src family kinases: defining the pathway(s)

• The morphogenetic consequences of cadherin-activated cell signaling and cooperatively with the actin cytoskeleton


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