

# IMB RESEARCHERS

The People & Their Passion



The highly integrated research environment at the IMB facilitates the fertile exchange of ideas and experimental approaches across the broad spectrum of molecular bioscience.

This enables a whole-of-system approach to understanding the basis of human and mammalian growth and development at the molecular, cellular and organ levels.

Only by understanding the complex molecular and cellular events that occur throughout a normal human life can scientists begin to understand

abnormalities responsible for many common human diseases and to find treatments for them. IMB researchers are particularly interested in the genetic programming of mammalian development and variation, the mapping of the structure, growth and dynamics of mammalian tissues and cells, and the development of new medicines and technologies.

This research will lead to new pharmaceuticals, gene therapies, technologies and diagnostics capable of identifying, halting or even reversing the progress of many diseases.

## Division of Genomics & Computational Biology

### RESEARCH FOCUS

This program includes the ARC Centre of Excellence in Bioinformatics and the Queensland Facility for Advanced Bioinformatics. It intersects with the Department of Mathematics and the School of Information Technology and Electrical Engineering. It focuses on understanding the genetic programming of humans, specifically, comparative mammalian and vertebrate functional genomics; genomics; and computational modelling of genetic and cellular regulatory networks (i.e. the Visible Cell™ project).

### Research Group Leaders

Tim Bailey  
Sean Grimmond  
John Mattick  
Mark Ragan  
Rohan Teasdale

## Division of Molecular Genetics & Development

### RESEARCH FOCUS

This program includes IMB's participation in the Cooperative Research Centre for Chronic Inflammatory Diseases; the Centre for Biotechnology and Development; and the NIH-funded project Nephrogenix, an initiative designed to develop new therapies for renal regeneration. It focuses on urogenital development, inflammation, cell signalling and cancer, molecular genetics and molecular biology of human diseases.

### Research Group Leaders

Peter Koopman  
Melissa Little  
George Muscat  
Andrew Perkins  
Rick Sturm  
Brandon Wainwright  
Carol Wicking

## Division of Molecular Cell Biology

### RESEARCH FOCUS

This program has received considerable support from the NANO Major National Research Facility; the Australian Cancer Research Foundation; Juvenile Diabetes Research Foundation International; and NIH. It is a major initiative of the IMB with the application of cryo-electron microscopy, cellular tomography, advanced visualisation and high-performance computing. It also includes the ARC Centre of Excellence in Bioinformatics. It focuses on the Visible Cell Project™; and cell architecture and trafficking.

### Research Group Leaders

John Hancock  
Brad Marsh  
Alan Munn  
Rob Parton  
Jennifer Stow  
Matt Sweet  
Michael Waters  
Alpha Yap

## Division of Chemical & Structural Biology

### RESEARCH FOCUS

This program has some of the most advanced equipment for structural biology in Australia, used in the development of new medicines and technologies, especially through exploration of Queensland's biodiversity. It has been responsible for a number of IMB spin-out companies based on new platform technologies for drug discovery, as well as developing novel drugs for human disease. It focuses on membrane protein structures; soluble protein and nucleic acid structures; and new drugs and therapies.

### Research Group Leaders

Paul Alewood  
Robert Capon  
David Craik  
David Fairlie  
Ben Hankamer  
Glenn King  
Richard Lewis  
Jenny Martin  
Mark Smythe

## Joint Appointments at the IMB

### RESEARCH FOCUS

The purpose of joint appointments is to foster collaborations in teaching, research and related activities between the IMB and Schools at The University of Queensland. Joint appointments involve a split of salary between the IMB and the relevant University of Queensland School and a joint appointee's commitment to the research and teaching activities at the IMB is greater than that of affiliate appointees. Joint appointees participate in all Institute activities including laboratory research, supervision of research higher degree students, and attendance at seminars, Divisional meetings and IMB Group Leader retreats.

### Research Group Leaders

Kevin Burrage  
Geoff Goodhill  
Alan Mark  
Geoffrey McLachlan

## Computational Cellular Biology

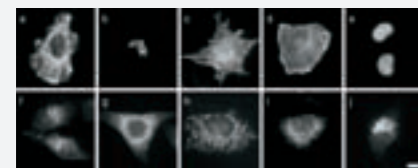


Rohan Teasdale

Individual cells contain a number of distinct sub-compartments, termed organelles. These organelles compartmentalise distinct biochemical pathways and cell-based physiological processes. Many proteins reside in one specific compartment while others are dynamically localised in multiple compartments. My research group is investigating how individual proteins are compartmentalised and defining the protein machinery responsible for their transport with a focus on the mammalian endosomal system.

Using a multidisciplinary approach combining computational biology with cell biology techniques, we investigate all aspects of this process. My research combines computational analysis across entire proteomes with focused investigation into individual proteins. Consequently, there are two overlapping streams of work:

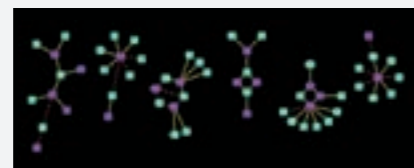
**Subcellular Localisation** - Determination of the subcellular localisation or compartmentalisation is an essential step in characterising the protein's function across all types of biomedical research, and its modulation needs to be considered when developing pharmaceutical agents. Our major long-term objective is to document the subcellular localisation of every protein within the mouse proteome. This will be achieved by a combination of experimental evidence, computational prediction and data mining.



**Endosomal Dynamics** - The endosomal/lysosomal system of mammalian cells is a highly dynamic organelle and the trafficking pathways within the endosomal system are fundamental for a wide variety of key cellular processes. My group is developing cellular and computational approaches to identify novel mammalian proteins associated with the endosomal system. A major current focus of the group is the characterisation of the mammalian retromer complex. We have implicated this complex, using real-time microscopy and molecular interaction techniques, in the sorting of numerous membrane receptors, including EGFR, within the endosomal system. With my group, Dr B. Collins has determined the high-resolution crystal structures of individual retromer proteins and is currently attempting to determine the structure of the entire complex. Currently we are undertaking a systems biology approach to examine the biogenesis of macropinosomes.

### RESEARCH PROJECTS

- Annotating the membrane organisation of mammalian secretory pathway proteins
- Maintaining and updating LOCATE: A Mouse Protein Subcellular Localisation Database - <http://locate.imb.uq.edu.au>



- Developing algorithms for prediction of protein features
- Developing computational approaches to analyse image and real-time microscopy data
- Studying endosome dynamics, macropinocytosis and retromer
- Investigating the systems biology of the mammalian endosome

### KEY PUBLICATIONS

Hamilton, N.A., Pantelic, R.S., Hanson, K., and Teasdale, R.D. (2007). Fast automated cell phenotype image classification. *BMC Bioinformatics* **8**: 110.

Sprenger, J. Fink, J.L., Karunaratne, S., Hanson, K., Hamilton, N., and Teasdale, R.D. (2007). LOCATE: A Mammalian Protein Subcellular Localisation Database. *Nucleic Acids Research* **36** (Database issue).

Aturaliya, R.N., Fink, J.L., Davis, M.J., Teasdale, M.S., Hanson, K.A., Miranda, K.C., Forrest, A.R.R., Grimmond, S.M., Suzuki, H., Kanamori, M., Kai, C., Kawai, J., Carninci, P., Hayashizaki, Y., and Teasdale, R.D. (2006). Subcellular Localisation of Mammalian Type II Membrane Proteins. *Traffic* **7**: 613-625.

Davis, M.J., Hanson, K.A., Clark, F., Fink, J.L., Zhang, F., Kasukawa, T., Kai, C., Kawai, J., Carninci, P., Hayashizaki, Y., and Teasdale, R.D. (2006). Differential use of endoplasmic reticulum signal peptides and transmembrane domains is a common occurrence within the variable protein output of transcriptional units. *PLoS Genetics* **2**: e46.

Kerr, M., Lindsay, M., Luetterforst, R., Hamilton, N., Simpson, F., Parton, R., Gleeson, P.A., and Teasdale, R.D. (2006). Visualisation of macropinosome maturation by the recruitment of sorting nexins. *Journal of Cell Science* **119**: 3967-3980.



### LAB MEMBERS

**Senior Research Officers:** Dr Brett Collins, Dr Nick Hamilton, Dr Zheng Yuan

**Research Officers:** Dr Lynn Fink, Dr Markus Kerr, Dr Stefan Maetschke, Dr Suzanne Norwood

**Research Assistants:** Seetha Karunaratne, Shane Zhang

**PhD Students:** Rajith Aturaliya, Melissa Davis, Daniel Shaw, Josefina Sprenger, Jack Wang

## How Genes Regulate Embryo Development



Peter Koopman

Our group specialises in studying genes controlling the formation of various organs in the developing embryo. In particular we are striving to understand the events that regulate the development of functional male and female gonads and the formation of the blood and lymphatic vessels.

The discovery of the gene SRY, which acts as a single switch to initiate the male pathway of development, was over a decade ago. However, few pivotal genes up- or down-stream of SRY have been identified since then, and the exact interactions and functions of those such as SOX9 and WT1 remain elusive. Our lab specialises in the identification and characterisation of genes in this pathway using techniques such as microarray screening and transgenic mouse models created via pronuclear injection, tetraploid aggregation and RNAi.

Of particular interest are those genes that shape the somatic cell environment of the gonad in addition to those that co-ordinate germ cell entry into mitotic arrest or meiosis. The recent discovery in our lab that retinoic acid controls germ cell meiosis entry in the female gonad has provided a pivotal point to understanding this process. Current projects are also focused on identifying the timing and mechanism of sex differentiation in the animal models of bovine and cane toads, in an effort to manipulate sex ratios and population numbers respectively.

A second major focus in our group includes investigating the function of Sox genes during embryo development. Specifically we are investigating the role of SOX18 in angiogenesis and

the formation of the lymphatic system.

The significant discovery that disruption of SOX18 leads to a delay of tumour formation has highlighted SOX18 as a potential target for antiangiogenic therapy of human cancers.

The study of embryo development gives us profound insight into mechanisms of disease and cancer. In particular, a detailed knowledge of sex determination will have vast biomedical significance, with up to 80 percent of human sex reversal cases currently unexplained. The use of new technologies and the availability of multiple species' genomes may allow us to better understand these cases, and aid in new therapies for patients. Our research also has the potential to assist the industrial sector through possible pest management and livestock sex-ratio manipulation contributing to the Australian economy and agricultural sectors.

### RESEARCH PROJECTS

- Understanding Sex Determination and Gonadal Development
- Studying the Development of Male Germ Cells
- Investigating Sox Gene Function and Evolution
- Studying the Molecular Genetics of Vascular and Lymphatic Development
- Developing Daughterless Cane Toads
- Triggering Male-Only Offspring Production in Beef Cattle

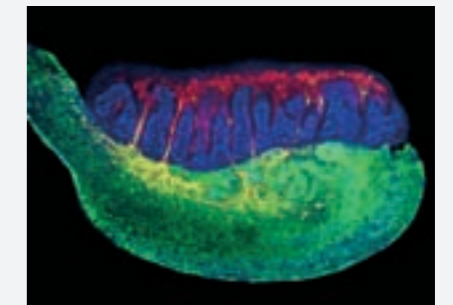
### KEY PUBLICATIONS

Wilhelm, D., Palmer, S., and Koopman, P. (2007). Sex determination and gonadal development in mammals. *Physiological Reviews* **87**: 1-28.

Bowles, J., Knight, D., Smith, C., Wilhelm, D., Richman, J., Mamiya, S., Yashiro, K., Chawengsaksophak, K., Wilson, M.J., Rossant, J., Hamada, H., and Koopman, P. (2006). Retinoid signaling determines germ cell fate in mice. *Science* **312**: 596-600.

Wilhelm, D., and Koopman, P. (2006). The makings of maleness: Towards an integrated view of male sexual development. *Nature Reviews Genetics* **7**: 620-631.

Young, N., Hahn, C.N., Poh, A., Dong, C., Wilhelm, D., Olsson, J., Muscat, G.E.O., Parsons, P., Gamble, J.R., and Koopman, P. (2006). Effect of disrupted SOX18 transcription factor function on tumor growth, vascularization, and endothelial development. *Journal of the National Cancer Institute* **98**: 1060-1067.



Recombinant organ culture with GFP expressing mesonephros (green) and wild type testis allows analysis of cell migration into the testis during development. Migrating endothelial cells integrate with endogenous vasculature (yellow and red respectively) which separate forming testis cords (blue).

### LAB MEMBERS

**Senior Research Officers:** Dr Josephine Bowles, Dr Catherine Browne, Dr Dagmar Wilhelm

**Research Officers:** Dr Annemiek Beverdam, Dr Mathias Francois, Dr Terje Svigen, Dr Brett Hosking, Dr Kallayane Chawengsaksophak

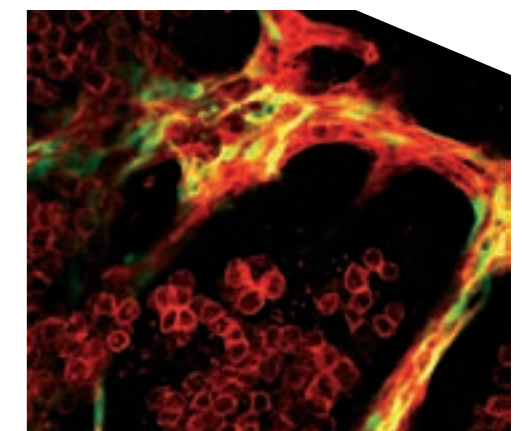
**Research Assistants:** Tara Davidson, Deon Knight, Desmond Tutt, Allen Feng, Vy Truong, Arief Mulyadi, Danielle Wilson

**Admin Assistant:** Rebekka van Kampen

**PhD Students:** Katherine Ewen, Juan Carlos Polanco, Stephen Bradford, Alexander Combes, Cassy Spiller, Diana Farkas, John Abramyan

**UROF Students:** James Holland

Platelet endothelial cell adhesion molecule (red) vividly outlines germ cell clusters and endothelial cells in a recombinant organ culture. Migrating endothelial cells marked by GFP (green) integrate into the endogenous vasculature to establish testis vasculature.



## Renal Development, Disease & Regeneration

Melissa Little



The central theme of this laboratory is the molecular basis of kidney development, disease and repair.

Each of us has a pair of kidneys that functions to excrete waste products in the form of urine. The kidneys therefore play an important role in maintaining fluid balance, blood volume and electrolyte balance. On top of this, they regulate blood pressure, bone density and number of red blood cells via the production of specific growth factors.

Chronic kidney disease (CKD) is a devastating disease and an expensive one to treat. Once this condition has reached end-stage renal failure, it can only be treated with dialysis or transplantation. Each year, more than 4000 Australian adults will be diagnosed with CKD, which cost the health system \$1.8 billion dollars in 2006. This cost is likely to escalate to \$4.7 billion by 2010. There is an urgent need to develop novel therapies as the rate of CKD is rising at 6-8 percent per annum, primarily due to increasing rates of Type II diabetes and obesity, and as only 1 in 4 patients will be lucky enough to receive a kidney transplant.

Our laboratory is acknowledged internationally for our work in defining the genes involved in normal kidney development and in dissecting the molecular basis of renal disease. The long-term aim of our laboratory is to develop novel cell-based or factor-based therapies for both acute and chronic kidney disease. Such therapies will grow out of our understanding of the processes involved in normal kidney development.

### RESEARCH PROJECTS

- Characterising the function of potential adult renal stem cells
- Characterising the cap mesenchyme on a molecular level
- Creating an atlas of gene expression during urogenital development
- Investigating the role of the resident tissue macrophage in renal regeneration
- Analysing the role of specific growth factors in renal development, repair and regeneration
- Screening the directed dedifferentiation of proximal tubule cells using lentiviruses
- Characterising the role of Crim1 in kidney and vascular development

### KEY PUBLICATIONS

Little, M., Brennan, J., Georgas, K., Davies, J., Davidson, D., *et al.* (2007). A high-resolution anatomical ontology of the developing murine genitourinary tract. *Gene Expression Patterns* **7**: 680-699.

Pennisi, D.J., Wilkinson, L., Kolle, G., Sohaskey, M.L., Gillinder, K., Piper, M.J., McAvoy, J., Lovicu,

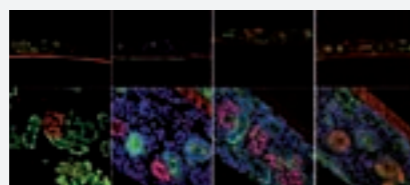
F., and Little, M.H. (2007). Crim1KST264/KST264 mice display a disruption of the Crim1 gene resulting in perinatal lethality with defects in multiple organ systems. *Developmental Dynamics* **236**: 502-511.

Rae, F., Woods, K., Sasmono, T., Campanale, N., Taylor, D., Ovchinnikov, D., Grimmond, S., Hume, D.A., Ricardo, S., and Little, M.H. (2007). Characterisation and trophic functions of murine embryonic macrophages based upon the use of a CSF-1R-EGFP transgene reporter. *Developmental Biology* **308**: 232-246.

Wilkinson, L., Gilbert, T., Pennisi, D., Challen, G., Ruta, L.-A., Kett, M., Cummings, M., and Little, M.H. (2007). Crim1KST264/KST264 mice implicate Crim1 in the regulation of VEGF-A activity during glomerular vasculature development. *Journal of the American Society of Nephrology* **18**: 1697-1708.

Challen, G.A., Bertonecello, I., Deane, J., Ricardo, S., and Little, M.H. (2006). Kidney side population cells represent a non-haematopoietic but heterogeneous population with multilineage and renal potential. *Journal of the American Society of Nephrology* **17**: 1896-1912.

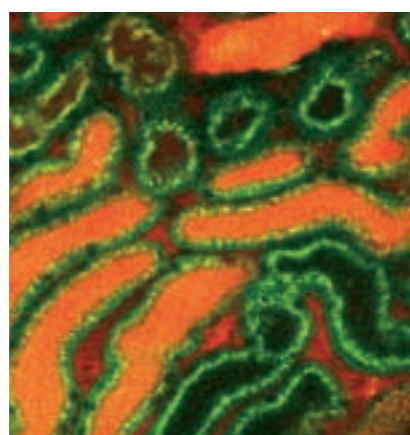
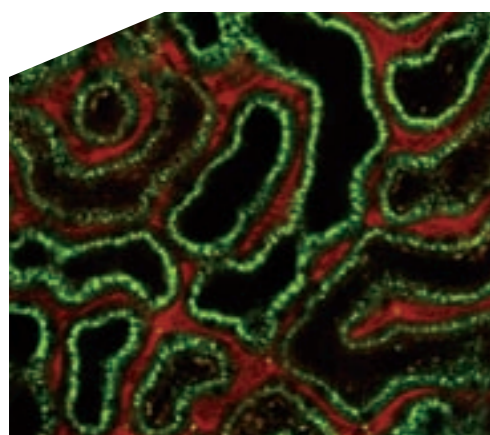
Little, M.H. (2006). Regrow or repair – potential regenerative therapies for the kidney. *Journal of the American Society of Nephrology* **17**: 2390-2401.



Reaggregation assay – proof of concept.



Live imaging (70kDa Rhodamine-dextran) of adult kidneys shows profound leakiness across the GBM.



### LAB MEMBERS

**Research Officers:** Dr Thierry Gilbert, Dr Lorine Wilkinson, Dr David Pennisi, Dr Fiona Rae, Dr Joan Li

**Research Assistants:** Kylie Georgas, Bree Rumballe, Jess Ineson, Emmanuelle Lesieur, Han Chui, Crystal McGirr

**PhD Students:** Genevieve Kinna, Michael Lusic, Caroline Hopkins

Rhodamine-dextran is evident in proximal tubules of KST264 adults.

## Nuclear Receptors, Skeletal Muscle & Metabolic Disease

George E.O. Muscat



Nuclear Hormone Receptors (NRs) control metabolism in metabolic, cardiovascular and endocrine organs. The importance of NRs in safeguarding human wellbeing is underscored by the curative efficacy of medicinals that target dysfunctional hormone signalling in the context of inflammation, cancer, endocrine and metabolic diseases. Nuclear hormone receptors function as agonist-dependent DNA-binding factors that translate nutritional (eg. dietary lipids), metabolic and pathophysiological signals into gene regulation. Proteins have been identified that belong to the NR superfamily on the basis of sequence identity, but the molecules that regulate their activity are unknown and they are denoted as orphan NRs. The orphans provide a platform for the unearthing of new signalling cascades that may have potential therapeutic utility.

Many orphan NRs are expressed in skeletal muscle, a peripheral tissue that accounts for ~40 percent of the total body mass and energy expenditure, and is a major site of fatty acid and glucose oxidation. Moreover, this lean tissue is involved in cholesterol efflux, expresses myostatin and cytokines that control inflammation, energy expenditure, lean body mass and adiposity. Consequently, muscle has a significant role in insulin sensitivity, the blood lipid profile, and energy balance. Therefore, the tissue has a notable role in the development of metabolic disease, and it is not surprising that NRs and skeletal muscle are emerging as targets in the battle against diabetes and obesity.

Surprisingly, the function of these orphan NRs in skeletal muscle metabolism has not been examined. The objective of our current research

is to examine the role of orphan NRs in skeletal muscle cell and animal models. We will test the hypothesis that the orphan NR4A and 1F subgroups regulate lipid and energy homeostasis in skeletal muscle. Recently, our group has provided evidence for regulatory crosstalk between beta-adrenergic and Nuclear Receptor (NR) 4A signalling in slow-twitch oxidative soleus muscle and fast-twitch glycolytic tibialis anterior muscle in the context of oxidative metabolism. The process involved PKA, MAPK and phosphorylation of CREB. Secondly, we have utilised several mouse models to demonstrate that NR1F subgroup is involved in the regulation of (i) serum and liver triglycerides and (ii) adiposity.

In addition, in collaboration with Dr Gary Leong (joint IMB/Mater Hospital) and Dr Edna Hardeman at Children's Medical Research Institute, Sydney, we are utilising the Ski transgenic mouse model to investigate the role of the ski gene in the metabolic changes associated with increased skeletal and decreased fat mass.

### RESEARCH PROJECTS

- Examining the role of the NR1D and F subgroups (Rev-erb and RORs) in lipid homeostasis and inflammation
- Elucidating the role of the NR4A subgroup (Nur77, NOR-1) in skeletal muscle energy balance and adrenergic signalling
- Determining the role and function of the Ski gene in body composition and metabolism via modulation of NR-dependent metabolism in skeletal muscle, fat and liver

### KEY PUBLICATIONS

Pearen, M.A., Myers, S.A., Raichur, S., Ryall, J.G., Lynch, G.S., and Muscat, G.E. (2008). The Orphan Nuclear Receptor, NOR-1, a Target of [beta]-Adrenergic Signaling, Regulates Gene Expression that Controls Oxidative Metabolism in Skeletal Muscle. *Endocrinology* Epub ahead of print

Raichur, S., Lau, P., Staels, B., and Muscat, G.E. (2008). Retinoid-related orphan receptor gamma regulates several genes that control metabolism in skeletal muscle cells: links to modulation of reactive oxygen species production. *Journal of Molecular Endocrinology* **39**: 29-44.

Smith, A.G., Luk, N., Newton, R.A., Roberts, D.W., Sturm, R.A., and Muscat, G.E. (2008). Melanocortin-1 receptor signalling markedly induces the expression of the NR4A nuclear receptor subgroup in melanocytic cells. *Journal of Biological Chemistry* Epub ahead of print

Myers, S.A., Wang, S.C., and Muscat, G.E. (2006). The chicken ovalbumin upstream promoter-transcription factors modulate genes and pathways involved in skeletal muscle cell metabolism. *Journal of Biological Chemistry* **281**: 24149-24160.

Pearen, M.A., Ryall, J.G., Maxwell, M.A., Ohkura, N., Lynch, G.S., and Muscat, G.E. (2006). The orphan nuclear receptor, NOR-1, is a target of beta-adrenergic signaling in skeletal muscle. *Endocrinology* **147**: 5217-5227.

Smith, A.G., and Muscat, G.E. (2006). Orphan nuclear receptors: therapeutic opportunities in skeletal muscle. *American Journal of Physiology: Cell Physiology* **291**: C203-17.

### LAB MEMBERS

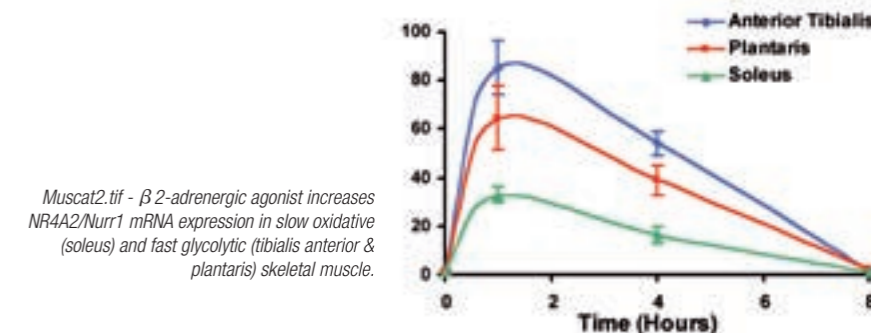
**Research Officers:** Dr Patrick Lau, Dr Stephen Myers, Dr Mary Wang, Dr Aaron Smith

**Research Assistants:** Rebecca Fitzsimmons, Rachel Burrow

**PhD Students:** Michael Pearen, Sathya Ramakrishnan, Suryaprakash Raichur, Lisa Crowther

Dr Gary Leong (Joint appointment Senior Research Officer, IMB, and Staff Specialist, Mater Childrens Hospital, NHMRC Clinical CDA & QLD Smart State Clinical Research Fellow)

**Research Assistant:** Nick Martel



## Blood Development

Andrew Perkins



Our group is interested in the transcriptional regulation mesoderm specification. We are primarily concerned with transcriptional hierarchies and how transcription factors work within biochemical and genetic pathways, and also how deregulation of such programs leads to cancer. Our group uses mouse and zebrafish model systems to examine gene function in vivo, and a wide variety of biochemical assays to examine gene function in vitro.

We have four primary focus areas:

1. Transcriptional hierarchies which are active during embryonic stem (ES) cell differentiation into mesoderm-derived tissues such as the kidney and blood. The methodologies used include: directed differentiation of ES cells in various recombinant growth factors, gene targeting and BAC recombineering for generating reporter ES cell lines and mice in which stem cells can be followed by epifluorescence and FACS, expression profiling and chromatin immuno-precipitation.

2. Transcriptional regulation of erythropoiesis. Mutations in the globin genes are the most common genetic mutations worldwide. These mutations are responsible for thalassaemia and sickle cell disease, which cause serious morbidity and mortality around the world. We are interested in trying to decipher the complex process of haemoglobin switching at a molecular level. The long-term goal is to design new drugs that target key regulators of this process and thereby reactivate foetal haemoglobin in adults.

3. Zebrafish are used as a vertebrate model for dissection of some of the earliest transcriptional events which underpin morphogenetic movements

which lead to the generation and 'education' of stem cells within the mesoderm germ layer. Once again we are concerned primarily with the activities of key 'master regulator' transcription factors of zinc finger and homeodomain classes. We have established expression profiling in zebrafish and have established assays and systems for study of morphogenesis.

4. The role played by the Kruppel-like factor (KLF) family of zinc finger genes in normal differentiation and human skin, colon and blood cancers.

### RESEARCH PROJECTS

- Studying transcriptional hierarchies active during ES cell differentiation into mesoderm-derived tissues
- Investigating the transcriptional regulation of erythropoiesis
- Studying morphogenesis using zebrafish models
- Investigating the role of KLFs in differentiation and cancer

### KEY PUBLICATIONS

Bruce, S.J., Gardiner, B.B., Burke, L.J., Cridland, S., Steptoe, A., Flanagan, J., Gongora, M., Grimmond, S.M., and Perkins, A.C. (2007). Dynamic transcription programs during ES cell differentiation towards mesoderm in serum versus serum-free (BMP4) culture. *BMC Genomics* **8**: 365.

Bruce, S.J., Rossiter, A.L., Steptoe, A.L., Busslinger, M., Bertram, J.F., and Perkins, A.C. (2007). A late Kidney Gene Expression Program within Mouse Embryoid Bodies. *Differentiation* **75**: 337-349.

Gardiner, M.R., Gongora, M.M., Grimmond, S.M., and Perkins, A.C. (2007). A global role for zebrafish *klf4* in embryonic erythropoiesis. *Mechanics of Development* **124**: 762-764.

Wilkins, S.J., Yoong, S., Verkade, H., Mizoguchi, T., Plowman, S.J., Hancock, J.F., Kikuchi, Y., Heath, J.K., and Perkins, A.C. (2007). *Mtx2* directs zebrafish morphogenetic movements during epiboly by regulating microfilament formation. *Developmental Biology* **314**: 12-22.

Hodge, D., Coghill, E., Maguire, T., Keys, J., Hartmann, B., Weiss, M., McDowall, A., Grimmond, S., and Perkins, A.C. (2006). A global role for EKLf in definitive and primitive haematopoiesis. *Blood* **107**: 3357-3370.

Papathanasiou, P., Perkins, A.C., Cobb, B.S., Ferrini, R., Sridharan, R., Hoyne, G.F., Nelms, K.A., Smale, S.T., and Goodnow, C.C. (2003). Widespread failure of hematolymphoid differentiation caused by a recessive niche-filling allele of the Ikaros transcription factor. *Immunity* **19**: 131-144.

Perkins, A.C., Sharp, A.H., and Orkin, S.H. (1995). Lethal  $\beta$ -thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLf. *Nature* **375**: 318-322.

### LAB MEMBERS

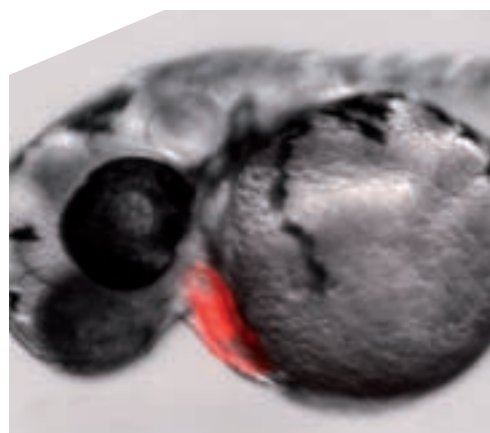
**Senior Research Officer:** Dr Janelle Keys (Cooley's Anemia Post-doctoral Research Fellow)

**Research Officers:** Dr Christine Neyt, Dr Stephen Bruce

**Senior Research Assistants:** Angela Lawton, Anita Steptoe

**Research Assistants:** Aliasha Griffin, Natalie Eriksson

**PhD Students:** Simon Wilkins, Simon Cridland, Melissa Gardiner, Michael Tallack, Paulo Amaral, Tom Whittington



## Human Pigmentation Genetics, Melanocyte Biology & Melanoma

Rick Sturm



The genetic and cellular understanding of human pigmentary traits is vital to assess an individual's response to sun exposure and their risk of skin cancer. We are investigating loci within the human genome that are responsible for an individual's pigmentation phenotype and identifying polymorphic alleles of these genes. A number of proteins essential to normal melanin biosynthesis have so far been identified and include the enzymes tyrosinase, tyrosinase-related protein-1 and dopachrome tautomerase (TYR, TYRP1 and DCT), while the P-protein (OCA2) is associated with blue eye colour, and the melanocortin-1 receptor (MC1R) is responsible for red hair colour. Furthermore, recent population-specific polymorphisms within the MATP (SLC45A2) and NCKX5 (SLC24A5) protein-coding regions have been correlated with the degree of pigmentation in human skin.

Our group has found that coding region variation within the MC1R gene can result in altered receptor activity and that this underlies the association with the red hair and fair skin phenotype (RHC). Nine common alleles have been studied and designated as either R (strongly associated with the RHC phenotype) or r (with lower penetrance). Using immunofluorescence and ligand binding studies, we have found that melanocytic cells expressing MC1R show strong surface localisation of the wildtype receptor but markedly reduced cell surface expression of some R variants. Moreover, MC1R variants can exert dominant negative activity on the wildtype receptor indicative of the ability of the receptor to homodimerize. We have also conducted genotyping studies to investigate the role of the OCA2 locus in inheritance of eye colour and other pigmentary traits associated with skin cancer risk in

white populations. SNPs spanning the OCA2 region were typed in a collection of 3839 adolescent twins, their siblings, and parents. The highest association for blue:non-blue eye colour was found for a major haplotype block mapping within the first intron of the OCA2 gene, notably this haplotype (TGT) representing 78.4 percent of alleles. The TGT/TGT diplotype found in 62.2 percent of samples was the major genotype seen to modify eye colour, with a frequency of 0.905 in blue or green compared to only 0.095 in brown eye colour.

Investigations into pigment cell biology have utilised cultures of both murine and human melanocytes, as well as numerous melanoma cell lines. We have published conditions for the isolation and propagation of human epidermal melanocyte precursors, termed melanoblasts, using medium supplemented with a range of growth factors and which differentiate into melanocytes upon mitogen withdrawal. Recent publications have suggested that melanoma may arise from the malignant transformation of melanocytic precursor cells residing in the skin. Our proposal is to study potential differences in the transcriptional and signalling network of skin-derived precursor (SKP) cells, when grown in vitro as spheroids and differentiated into melanocytes. We aim to identify the differentiation and regulatory pathways active in normal melanocyte growth that differ to those responsible for melanoma development, and formation of spheroids from melanoma cell lines.

### RESEARCH PROJECTS

- Understanding skin cancer risk phenotypes through studying the interaction of genes involved in skin, hair and eye colour

- Undertaking parallel genetic and cellular analysis of human melanogenesis
- Investigating eye colour as a genetic trait
- Researching melanocytic spheroids as a model for melanoma development and metastasis

### KEY PUBLICATIONS

Beaumont, K.A., Shekar, S.N., Newton, R.A., James, M.R., Stow, J.L., Duffy, D.L., and Sturm, R.A. (2007). Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. *Human Molecular Genetics* **16**: 2249-2260.

Duffy, D.L., Montgomery, G.W., Chen, W., Zhao, Z.Z., Le, L., James, M.R., Hayward, N.K., Martin, N.G., and Sturm, R.A. (2007). A three-SNP haplotype in the intron 1 of OCA2 explains most human eye color variation. *American Journal of Human Genetics* **80**: 241-252.

Sturm, R.A. (2006). A golden age of human pigmentation genetics. *Trends in Genetics* **22**: 464-468. (Journal Cover)

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Duffy, D.L., Box, N.F., Chen, W., Palmer, J.S., Montgomery, G.W., James, M.R., Hayward, N.K., Martin, N.G., and Sturm, R.A. (2004). Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Human Molecular Genetics* **13**: 447-461. (Journal Cover)

Sturm, R.A., and Frudakis, T.N. (2004). Eye colour: portals into pigmentation genes and ancestry. *Trends in Genetics* **20**: 327-332. (Journal Cover)



### LAB MEMBERS

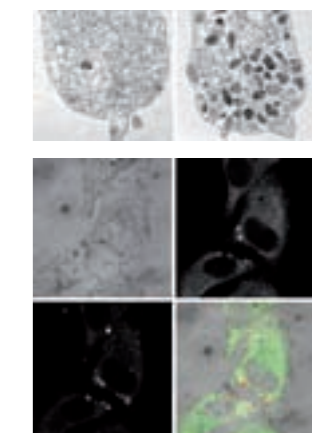
**Research Officer:** Dr Anthony Cook

**Research Assistants:** Darren Smit, Caroline Sturm, Amy Thurber

**PhD Students:** Helene Johanson, Luke Kirkwood, Don Roberts, Kimberley Beaumont

**Honours Student:** Wen Lim

**Undergraduate Student:** Poh Yuen Chin



Melanocytes and melanosomes.

## Tissue Repair & Cancer



Brandon Wainwright

Using genomic approaches our group mapped and isolated genes affecting two human genetic conditions, cystic fibrosis (CF) and naevoid basal cell carcinoma syndrome (NBCCS). From this work has emerged a focus upon the pathways that lead to inflammation, tissue repair and proliferation in the context of these two diseases. In particular the patched gene, discovered from our studies on NBCCS, has defined a signalling pathway known as the ("hedgehog pathway") which appears to be mutated or perturbed in a wide range of tumour types, including lung, gastro-intestinal, skin, pancreatic, prostate, brain and ovarian cancer. This has led us to focus on the role of hedgehog signalling, not only in cancer but also on the regulation of stem cell compartments. Increasingly it appears that in some tumour types there are cells known as "cancer stem cells" which reside within the tumour and are responsible for the overall phenotype. Currently such cells can be partially defined functionally but their molecular signature remains elusive. We believe that the patched/hedgehog pathway defines many of the characteristics of such stem cells and is a powerful starting point for understanding tumour biology and the development of new therapeutics.

Given that cancer represents a state of unregulated cell growth, it is likely that the pathways that lead to cancer are also involved in the normal process of tissue growth and repair. Several of our studies are particularly directed at the role of the hedgehog (and other pathways) in repair and regeneration. In our laboratory this is a developing theme and focused upon the lung. From our studies on cystic

fibrosis we are gaining an understanding of how infection and inflammation in this disorder damage the lung epithelium and severely compromise lung function. At the same time, in order to provide new therapeutic avenues, we are analysing the molecular signature of repair of the lung epithelium using the patched/hedgehog pathway as a start point. The processes of inflammation, damage, repair and cancer are intimately connected and to gain an insight into one process enables progress in all to be made. This will lead us to a better understanding of how cell-based therapies might be used to treat lung diseases as well as likely provide valuable insights into the mechanism of lung cancer.

As part of our experimental approach our laboratory makes extensive use of transgenic and knockout mice. However at all points we refer back to the human diseases under study and have major activities based around mutation analysis, transcriptomics and proteomics of human material, integrating the data from all systems.

As a result of these studies we have a particular interest in the interface between developmental cell biology and human genetics, and in therapeutic interventions such as gene or cell therapies.

### RESEARCH PROJECTS

- Controlling neuronal stem cells and CNS by the patched/hedgehog pathway
- Investigating the molecular basis of primary brain tumours

- Controlling the stem cell niche in mammalian epidermis and skin cancer
- Studying infection, inflammation and repair in cystic fibrosis mice and cystic fibrosis infants
- Controlling lung regeneration following injury

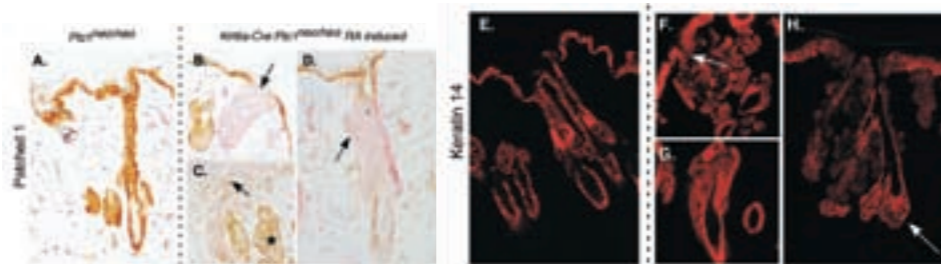
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Ingram, W., McCue, K., Tran, T., Hallahan, A., and Wainwright, B. (2007). Sonic hedgehog regulates Hes1 through a novel mechanism that is independent of canonical Notch pathway signalling. *Oncogene* **27**: 1489-1500.

McMorran, B., Patat, S., Carlin, J., Grimwood, K., Jones, A., Armstrong, D., Galati, J., Cooper, P., Byrnes, C., Francis, P., Robertson, C., Hume, D., Borchers, C., Wainwright, C., and Wainwright, B. (2007). Novel neutrophil-derived proteins in bronchoalveolar lavage fluid indicate an exaggerated inflammatory response in pediatric cystic fibrosis patients. *Clinical Chemistry* **53**: 182-91.

Adolphe, C., Hetherington, R., Ellis, T., and Wainwright, B. (2006). Patched1 functions as a gatekeeper by promoting cell cycle progression. *Cancer Research* **66**: 2081-2088.

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Loss of patched leads directly to skin tumours.

### LAB MEMBERS

**Senior Research Officer:** Dr Tammy Ellis

**Research Officers:** Dr James Palmer, Dr Richa Dave, Dr Elaine Costelloe

**Research Assistants:** Ailsa McCormack, Melissa Bourboulas

**PhD Students:** Karen McCue, Rehan Villani, Uda Ho, Elaine Haase, Jonathon Robson, Lena Constantin, Azita Ahadzadeh

**Masters Student:** Ann-Marie Michalski

**Honours Student:** Larissa Upward

## Developmental Genes & Human Disease



Carol Wicking

Defects arising from abnormal embryonic development are a major cause of infant mortality and childhood disability. Many such disorders are characterised by anomalies of the limbs and craniofacial region, suggesting a conservation of the molecular development of these structures. Using the mouse as a model system, we aim to identify and characterise novel molecules contributing to the development of the limb and face, with particular emphasis on genes regulated by the Hedgehog signalling pathway. Because of the importance of Hedgehog and other developmental signalling pathways in tumorigenesis, many of these genes will also be important in cancer.

Using genomics-based approaches we have identified a number of novel or poorly characterised genes with potential roles in embryonic development and disease. For those genes of interest we are undertaking a more detailed characterisation at both the cell and whole-organism level. We employ standard cell biology and biochemical techniques to shed light on the cellular role of these molecules, and in some cases are using transgenic or knockout approaches in the mouse to elucidate function. We are currently pursuing analysis of a protein that we have shown regulates cell migration. Our ultimate aim is to correlate the genes we identify with human disease, and we are currently analysing a number of genes for a role in tumour formation and/or progression.

The limb bud has long been considered a paradigm for analysis of embryonic development, and Hedgehog signalling is a key determinant of

patterning in the vertebrate limb. We are therefore using a number of mouse models of Hedgehog signalling to further explore the function of this pathway in limb development.

### RESEARCH PROJECTS

- Studying conditional knockout of the Hedgehog receptor patched in the developing mouse limb
- Identifying and analysing genes regulated by the transcription factor Gli3 in the developing limb
- Identifying and analysing genes expressed in the facial primordia
- Analysing a novel regulator of cell migration on a cellular level

### KEY PUBLICATIONS

Bennetts, J.S., Rendtorff, N.D., Simpson, F., Tranebjaerg, L., and Wicking, C. (2007). The coding region of *TP53INP2*, a gene expressed in the developing nervous system, is not altered in a family with autosomal recessive non-progressive infantile ataxia on chromosome 20q11-q13. *Developmental Dynamics* **236**: 843-852.

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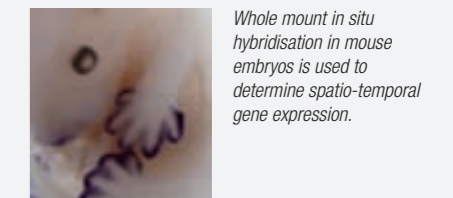
Hollway, G.E., Maule, J., Gautier, P., Evans, T.M., Keenan, D.G., Lohs, C., Fischer, D., Wicking, C., and Currie, P.D. (2006). Scube2 mediates Hedgehog signaling in the zebrafish embryo. *Developmental Biology* **294**: 104-118.

Simpson, F., Lammerts van Bueren, K., Butterfield, N., Bennetts, J.S., Bowles, J., Adolphe, C., Simms, L.A., Young, J., Walsh, M.D., Leggett, B., Fowles, L.F., and Wicking, C. (2006). The PCNA-associated factor KIAA0101/p15PAF binds the potential tumour suppressor product p33ING1b. *Experimental Cell Research* **312**: 73-85.

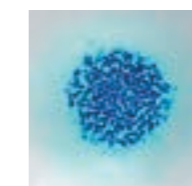
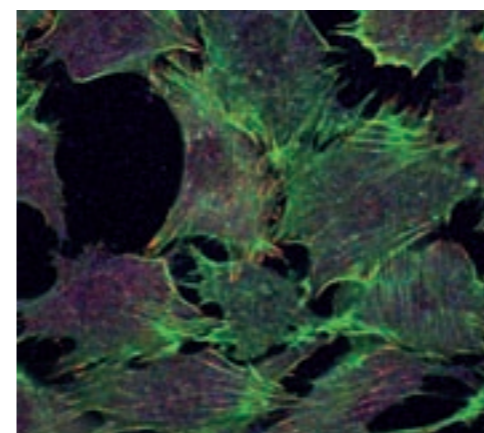
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Whole mount in situ hybridisation in mouse embryos is used to determine spatio-temporal gene expression.



High density micromass culture established from limb mesenchymal cells and stained with Alcian blue to detect sulfated proteoglycans associated with cartilage. This method is used to study chondrogenesis in vitro.

Immunofluorescence analysis to reveal subcellular localisation of proteins can provide insight into function.

### LAB MEMBERS

**Senior Research Officer:** Dr Fiona Simpson (RD Wright Fellow)

**Research Officer:** Dr Kerry Mantou

**Research Assistant:** Vicki Metzlis

**PhD Students:** Natalie Butterfield, Liam Town

**Honours Student:** Amanda Bain

**Visiting Student:** Diane Schlupe (Netherlands)