EASTBIO Annual Symposium 2014
POSITIONING BIOSCIENCE RESEARCH FOR THE FUTURE

Abstracts Booklet
EASTBIO Student Presentations (2012 & 2013 cohorts)

University of Dundee
Dalhousie Building, 28-29 August 2014
Welcome to the EASTBIO Annual Symposium 2014

A very warm welcome to the 2014 Annual Symposium of the BBSRC-funded EASTBIO Doctoral Training Partnership. The Annual Symposium represents one of the highlights in the EASTBIO calendar and celebrates research in bioscience presented by our current postgraduate students and in plenary talks by key researchers encompassing the whole breadth of EASTBIO’s research themes.

The theme of this year’s conference is ‘Positioning bioscience research for the future’. Against the big challenges facing biosciences today, the EASTBIO community will use this symposium to interact and engage on bioscience approaches and achievements that respond to contemporary key issues. This conference provides our students with a fine opportunity to present their research projects spanning from healthy ageing to biotechnology to food security and to discuss the progress of their work with EASTBIO supervisors and experts in the field.

In this booklet, you will find the abstracts for poster presentations and PechaKucha 20X20 presentations by the two cohorts of EASTBIO students.

Dr Arno Muller

On behalf of the EASTBIO Management Group and the Symposium host institution
Acknowledgment: The work published here was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) -- grant number BB/J01446X/1.
Contents

About the EASTBIO Annual Symposium 2014 ................................................................. 2

Part I. Presentations by EASTBIO second-year students, by theme .................................. 5
  Presentations for Basic Bioscience underpinning Health (Ageing) ...................................... 7
  Presentations for Bioenergy & Industrial Biotechnology .................................................... 16
  Presentations for Food Security ...................................................................................... 21
  Presentations for World Class Bioscience ...................................................................... 27

Part II. Poster session by EASTBIO first-year students, by theme .................................. 38
  Poster session for Basic Bioscience underpinning Health (Ageing) .................................... 41
  Poster session for Bioenergy & Industrial Biotechnology .................................................. 51
  Poster session for Food Security ..................................................................................... 58
  Poster session for World Class Bioscience ..................................................................... 73
## Part I. Presentations by EASTBIO second-year students, by theme

### Presentations for Basic Bioscience underpinning Health (Ageing) * 29 August, 10.10-12.20

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Title of presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle, Andrew</td>
<td>Elucidating downstream pathways and processes regulated by the cellular prion protein</td>
</tr>
<tr>
<td>Diamond, Alexandra</td>
<td>Roles of axon guidance molecules in developing limb joint formation</td>
</tr>
<tr>
<td>De Mello, Vanessa</td>
<td>The Hippo Pathway during muscle development and ageing</td>
</tr>
<tr>
<td>Guest, Patrick</td>
<td>The ABAD enzyme as a therapeutic target in the Alzheimer's diseased brain</td>
</tr>
<tr>
<td>Houston, Dean</td>
<td>Investigating the interplay between PHOSPHO1 and SMPD3 in the initiation of skeletal mineralisation</td>
</tr>
<tr>
<td>McLean, Fiona</td>
<td>A proteomic study linking diet, ageing and cognition</td>
</tr>
<tr>
<td>Sivakumaran, Magali</td>
<td>Out with the old, in with the new: Novelty judgements as a translational tool to assess healthy ageing</td>
</tr>
</tbody>
</table>

### Presentations for Bioenergy & Industrial Biotechnology * 29 August, 14.55-16.40

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Title of presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgiou, Charis</td>
<td>From MD to X-ray crystallography: Rational design of isoform specific ligands</td>
</tr>
<tr>
<td>Lamont, Ciaran</td>
<td>Biohydrogen as a fuel: Understanding and engineering bacterial hydrogenase enzymes for white biotechnology applications</td>
</tr>
<tr>
<td>McLean, Chris</td>
<td>Human SK1 and S1P(_2); Studies of the enzymes that control S1P levels (PhD Project title)</td>
</tr>
<tr>
<td>Slikas, Justinas</td>
<td>DNA Gears and Axles</td>
</tr>
<tr>
<td>Venkateswaran, Seshasailam</td>
<td>Bacteria repellent coatings for biomedical devices</td>
</tr>
</tbody>
</table>

### Presentations for Food Security * 28 August, 13.10-15.00

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Title of presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnbrook, Matthew</td>
<td>Trivial Hirsuit: Finding the Hairy gene in <em>Antirrhinum</em></td>
</tr>
<tr>
<td>Dalby, Matthew</td>
<td>Investigation of the Interactions Between Natural Products and Pathogenicity in the Fish Pathogen Yersinia ruckeri (PhD project title)</td>
</tr>
<tr>
<td>Name</td>
<td>Title</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ireland, Kirsty</td>
<td><em>In vitro</em> modelling of the relationship between amyloid seeding and plaque formation</td>
</tr>
<tr>
<td>Patil, Vrushali</td>
<td>Effect of HvAPETALA2 on internode development</td>
</tr>
<tr>
<td>Sehgal, Anuj</td>
<td>M cells, gateway to infection or gatekeepers of immunity?</td>
</tr>
<tr>
<td>Turnbull, Matthew</td>
<td>The role of the A- and B- alleles of the influenza A virus NS segment in host adaptation</td>
</tr>
</tbody>
</table>

**Presentations for World Class Bioscience** *28 August, 10.10-12.05 & 29 August, ~15.40-16.40*

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giotti, Bruno</td>
<td>Analysis and modelling of the mammalian cell cycle pathway (PhD Project title)</td>
</tr>
<tr>
<td>Magill, David</td>
<td>Modulation of Fatty-Acid Induced Skeletal Muscle Inflammation by the Peripheral Endocannabinoid Sys</td>
</tr>
<tr>
<td>McFarland, Matthew</td>
<td>Using Ribosomal feedback loops to control gene expression in synthetic biology circuitry</td>
</tr>
<tr>
<td>McGarvey, Alison</td>
<td>Global gene expression analysis in elucidating the molecular characteristics of emerging hematopoietic stem cells</td>
</tr>
<tr>
<td>Mitchell, Elaine</td>
<td>Spatial stochastic modelling of the NFκB gene regulatory network</td>
</tr>
<tr>
<td>Parkin, Hannah</td>
<td>Analysis of heparan sulfate function in the developing mammalian forebrain using systematic gene expression analysis</td>
</tr>
<tr>
<td>Picton, Laurence</td>
<td>From neurons to behaviour: the control of tadpole swim networks by dopamine</td>
</tr>
<tr>
<td>Tennant, Sarah</td>
<td>The role of grid cells in path integration</td>
</tr>
<tr>
<td>Tillotson, Rebekah</td>
<td>Are the abilities to bind methylated DNA and the NCoR/SMRT repressor complex sufficient for MeCP2 function?</td>
</tr>
<tr>
<td>Tzioutziou, Nikoleta</td>
<td>Effect of low temperature on the alternative splicing of Arabidopsis thaliana circadian clock genes</td>
</tr>
<tr>
<td>Younger, Katie</td>
<td>Control of apoptosis in response to mitotic arrest</td>
</tr>
</tbody>
</table>
The cellular prion protein, PrP\textsuperscript{C}, has been associated with a diverse array of cellular functions, including protecting cells from apoptosis and oxidative stress, maintaining mitochondrial function, regulating calcium homeostasis, and modulating neuronal excitability. We are investigating the molecular mechanisms regulated by PrP\textsuperscript{C}, which will improve our knowledge of its putative stress-protective functions and of how these could be harnessed therapeutically. Firstly, we have stably transfected the prion protein gene into SH-SY5Y neuroblastoma cells that lack detectable endogenous PrP\textsuperscript{C}. Using monoclonal lines derived from these cells, we have shown that PrP\textsuperscript{C} expression seems to confer some protection against the toxins paraquat and staurosporine, as measured by survival and cytotoxicity assays. This protective effect, however, seems to be clone-dependent, suggesting that the site of integration of the expression plasmid into the cellular DNA is of some importance. Subsequently, we plan to study how PrP\textsuperscript{C} influences responses to these toxic insults, and others, at a molecular level; this work will focus specifically on the expression levels of targets identified from proteomic analyses of PrP\textsuperscript{C}-expressing SH-SY5Y cells, as well as additional targets confirmed as being differentially expressed in the brains of PrP\textsuperscript{C}-null mice. Lastly, we have used confocal microscopy to visualize internalization of extracellularly applied, fluorescently tagged recombinant PrP by SH-SY5Y cells. This work aims to uncover the intracellular trafficking pathways that the normally cell surface-anchored PrP\textsuperscript{C} follows upon internalization.
Slits/Robos, F-spondin and Dscam have been identified as axon guidance molecules that direct axons along specific pathways to their final targets through attraction or repulsion. Mutations in axon guidance molecules Slits/Robos and Dscam have been linked to spinal disorders, such as scoliosis and kyphosis, in human and animal models. Furthermore, an increased expression of F-spondin is seen in cartilage of human osteoarthritic joints. Axon guidance molecules are clearly essential for normal joint development and maintenance, though our understanding of their roles in these processes is rudimentary. I aim to identify the functions of axon guidance molecules during skeletal development. I have ascertained the normal expression patterns of axon guidance molecules and their receptors, including Slits/Robos, Dscam and F-spondin, and seen that they are expressed at high levels in developing joints of mouse embryos. To begin to address their function in joint development I am performing functional studies utilising chick models and transgenic mice to deduce how mutations and misexpressions affect joint development. The relevance of the results obtained to human disease and strategies aimed at their treatment will also be explored.
The Hippo pathway has been shown to regulate organ size and cell number and when uncontrolled can lead to cancer. Furthermore there is emerging evidence that the Hippo pathway can regulate muscle stem cell fate.

Downstream members regulated by the Hippo core kinases are transcriptional co-activators Yap, Taz and Vestigial like proteins 1-4. When active these bind Tead transcription factors 1-4 that activate muscle regulatory genes via MCAT elements. The first aim of my research is to understand how these downstream elements regulate muscle stem cell fate. To start addressing this, expressional levels were measured in a C2C12 cell culture model and an *in vivo* regeneration model. Results demonstrated some members such as Yap to be more abundant during proliferation and others such as Taz to be more abundant during differentiation. This leads us to hypothesise that certain complexes may form to target certain MCAT elements involved in either proliferation or differentiation. Additional studies are required to see how these interact; this work also includes using the developing Chicken as a model. To map endogenous expression Chicken Yap1 *in situ* probes have been designed as well as constitutively active HYAP1-S127A RCAS plasmid; work is still on going.

Additionally studies suggest that muscle stem cells proliferate more during ageing, possibly due to increased maintenance. Therefore it can be predicted that this increased proliferation may be regulated by the Hippo pathway. Protein lysates were extracted from aged muscle gastrocnemius and western blots against Yap and Taz were carried out. Results obtained for Yap and Taz showed no difference during ageing, further testing is required.
Alzheimer's disease is the most common form of senile dementia and as such represents a severe burden, both financially and socially to the global population. Current therapies against this disorder are at best palliative, managing symptoms but failing to address the underlying cause and thus disease progression and patient death remain a certainty. There is clearly an urgent need for new, more effective, therapeutic agents against this disease. Despite being first identified over a century ago and subject to intensive research, the pathogenic mechanisms of Alzheimer's disease remain poorly understood, with proponents for a number of theories. Arguably the most widely accepted of these, the amyloid cascade hypothesis, proposes that an abnormal accumulation of amyloid beta peptide (Aβ) is the key disease causing event, perturbing normal cell functions and triggering a cascade of events resulting in the synaptic dysfunction and neuronal loss characteristic of the disease. In recent years there has been a shift of focus from the extracellular plaques formed by Aβ, to soluble intracellular forms and their binding partners. The amyloid binding alcohol dehydrogenase enzyme (ABAD) is one such binding partner. The interaction between ABAD and Aβ has been shown to be cytotoxic, as shown by reactive oxygen species production and cytochrome c mobilisation. Interestingly, it appears that the ABAD enzyme must be catalytically active for this oxidative stress to be observed. Therefore, the direct inhibition of the ABAD enzyme may offer a novel therapeutic approach to treating Alzheimer's disease, possibly reducing Aβ associated oxidative stress and thus protecting against neuronal loss.
DA Houston*[1] Investigating the interplay between PHOSPHO1 and SMPD3 in the initiation of skeletal mineralisation

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PHOSPHO1 is a bone-specific phosphatase essential for the initiation of skeletal mineralisation. Ablation of Phospho1 results in severe hypomineralisation. Hypomineralisation of the skeleton is also observed in mice deficient in sphingomyelin phosphodiesterase 3 (SMPD3). SMPD3 cleaves sphingomyelin to generate ceramide and phosphocholine. Interestingly, PHOSPHO1 shows high substrate-specificity towards phosphocholine and therefore we hypothesise that the co-ordinated actions of SMPD3 and PHOSPHO1, through the generation and processing of phosphocholine, respectively, are vital for matrix mineralisation. This present study sought to uncover the interplay between these regulators of skeletal mineralisation.

In phosphatase substrate-free conditions (no βGP), MC3T3-Clone14 osteoblast-like cells exhibited profound matrix mineralisation by day-10 of culture compared to similarly treated MC3T3-Clone24 cultures (p<0.01). Furthermore, there was an increase in Phospho1 (8-fold, p<0.001) and Smpd3 (3.5-fold, p<0.001) expression in the Clone14 compared to the Clone24 cells. This elevation of PHOSPHO1 and SMPD3 expression was confirmed at the protein level. Phospho1−/− osteoblasts, likewise showed decreased mineralisation at 28-days in culture compared to wild-type cells (p<0.001). Wild-type E15 metatarsals mineralised their diaphysis over a 5-day culture period and showed an increase in Phospho1 expression at day-5 compared to day-0 metatarsals (9.4-fold, p<0.01). In contrast Phospho1−/− metatarsals disclosed minimal mineralisation. Moreover, Smpd3 expression was higher in wild-type compared to Phospho1−/− metatarsals at day-5 (2-fold, p<0.05)

Matrix mineralisation was appreciably decreased in cultures with reduced or absent Phospho1 expression. Diminished or absent Phospho1 expression was associated with reduced Smpd3 expression suggesting the existence of regulatory negative feedback mechanisms in the provision of PHOSPHO1 substrates for skeletal mineralisation.
Fig. 1.

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<thead>
<tr>
<th>Days in culture</th>
<th>Wild-Type metatarsals</th>
<th>Phospho1^-/^- metatarsals</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

500 µm
Obesity and type 2 diabetes are associated with an increased risk of dementia\(^1\),\(^2\). A high-fat diet (HFD) causes memory impairment in rodents; however, complex episodic-like memory has not been tested in this context. Episodic memory is the recollection of events using a “what-where-when/which” experience and is compromised in neurodegenerative diseases including Alzheimer’s disease\(^3\),\(^4\),\(^5\).

To identify a link between a HFD and episodic memory loss, 12 week old, male, C57Bl/6 mice, were fed either a HFD (60% energy from fat) or a low fat diet (LFD) (10% energy from fat) ad libitum and tested for 2 weeks with an object-place-context (OPC) recognition memory task. This task challenges episodic-like hippocampal dependent memory in rodents, with rats bearing hippocampal lesions and the 3xTgAD mouse model of Alzheimer’s disease being impaired in this task\(^6\),\(^7\). To test glucose tolerance a separate group of mice underwent intraperitoneal glucose tolerance tests (IPGTTs). We found that glucose tolerance and episodic-like memory are impaired after only days of a HFD and these deficits continued throughout the testing period. This indicates that both hippocampal function and glucose tolerance are compromised rapidly by HFD. These data link HFD to the rapid induction of glucose intolerance and memory deficit and have implications for the link between diet, obesity and cognitive decline.

References:


As we age in later adulthood, even healthily, our memory tends to decline: we forget names, what we were going to say, even sometimes our address or a family member. This brings with it various consequences, from feeling frustrated or self-conscious, to possibly needing full time care. We are an aging population and living longer, so these are important concerns. Much research tries to better understand this memory decline to potentially tackle these issues. A lot of this research often assumes memory deficits occur because we “forget” - we mistake familiar things as “new”. For example, when we praise our neighbour on their new ornament and they tell us that we saw it last week, making the same remark, we have mistaken an object that is familiar for a “new” object. However, there is new research which suggests that maybe our memory decline in older age is due to our brains not treating new things as “new”. The ability to detect that something is new is an important aspect of memory, one which is often overlooked. When we see something new we are programmed to learn about and store it, so that we can retrieve this from memory when required. If our brain doesn’t fully recognise something new then we may not store it, and hence we won’t be able to remember or recognise it in future because it has not been stored. Therefore I will discuss my research which looks at whether we process novelty and familiarity in the same way.
Cyclophilins are proteins able to catalyze the interconversion of trans/cis isomers of proline and belong to the peptidyl-prolyl isomerases family (PPIase).

In addition to their PPIase activity, cyclophilins have diverse biological roles and have been implicated in a number of different diseases such as HIV-1 and HCV. Although several cyclophilin inhibitors have been reported in the literature, none are able to inhibit with high specificity selected cyclophilin isoforms.

To facilitate the development of isoform-specific cyclophilin ligands, we are pursuing detailed studies of cyclophilin dynamics and binding thermodynamics using molecular simulations, biophysical assays and protein X-ray crystallography. Initial efforts are focussed on free energy calculations of cyclophilin A (CypA) in complex with a novel class of small molecule inhibitors. Analogues of the original hit compound, for which binding affinities were reported from Surface Plasmon Resonance (SPR) experiments, were designed, purchased and tested using X-ray crystallography.

Models of the apo and holo CypA were set up using the Amber and AmberTools software and free energy calculations were performed using Sire/OpenMM. Intermolecular interactions of CypA complexes observed from X-ray crystallography and MD simulations have been characterised. Those interactions include: hydrogen bonding interactions between the ligand and protein, interactions with water molecules. Efforts towards the optimisation of the hit compounds and the computation of their binding free energies using other biophysical methods will be reported.

References:


(3) www.siremol.org.


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Hydrogen has long been seen as an ideal alternative to fossil fuels, due to its high energy-to-mass ratio and because greenhouse gases are not produced during its combustion. However, the current technologies used in both the production and consumption of hydrogen fuel at present are not appropriate for scaling up to the level required that would allow hydrogen to entirely replace fossil fuels. Thus, research is being carried out to find alternative ways to produce the gas.

Biohydrogen, that is molecular hydrogen derived from biological processes, is one possible solution. Not only does biohydrogen possess the benefits associated with H₂ fuel consumption described above—namely the high energy yield and innocuous end product (water) — but it is also a truly renewable fuel due to its biological origins. As such, there is currently a great deal of research with regards to trying to allow for the production of biohydrogen on an industrial scale. Such work is centred on hydrogenase enzymes, which catalyse the reversible oxidation of dihydrogen (H₂) to protons and electrons. Despite accounting for the majority of biohydrogen production in organisms, native hydrogenase activity is too low to allow for direct utilisation in industrial scale hydrogen production. Research is therefore being carried out to attempt to engineer hydrogenases to improve their activity, and this is one aspect of my PhD project.

A second aspect of my project relates to the fact that such work on hydrogenases is currently limited to so-called reverse genetic approaches through targeted mutagenesis, which is not only time-consuming, but technically challenging and limited in scope. The ability to employ forward genetic approaches via random mutagenesis would revolutionise this field of research, however this necessitates the development of a screen to measure hydrogenase activity \textit{in vivo}. With this in mind, much of my work has been centred on the construction of an intracellular ‘Hydrogen Biosensor’.
In recent decades, DNA has gained attention not only as an information-coding molecule, but also as a structural element (Seeman, 1982). The DNA nanotechnology field has evolved rapidly and remarkable research into structural exploitations have been made, e.g. DNA origami (Rothemund, 2006). Devices and machines, capable of programmable action have been described and constructed, such as the bipedal DNA walker and DNA tweezers (Turberfield et al., 2000, 2004).

Here we describe a DNA system of interlocked single-stranded DNA rings that roll against each other, driven by a DNA polymerase. A single-stranded DNA rotaxane is proposed as means of immobilising and exploiting the interlocked circles structure for further applications in DNA nanotechnology.

We show preliminary evidence of a supramolecular assembly being formed. Rolling circle amplification assay to monitor the action of this motor is described and tested. Future developments of functionalisation, visualisation and applications of the structure are addressed. For example, the system can be used as an integral part in larger assemblies of nanomechanical devices performing autonomous, programmable action.
Nosocomial infections due to bacteria have serious implications on the health and recovery of patients in a variety of medical scenarios. Since bacterial contamination on medical devices contributes to the majority of nosocomial infections, there is a need for redesigning the surfaces of medical devices, such as catheters and tracheal tubes, to resist the binding of bacteria. In this work, polyurethanes and polyacrylates/acrylamides, which resist binding by the major bacterial pathogens underpinning implant-associated infections, were identified using high-throughput polymer microarrays. Subsequently, two ‘hit’ polymers, PA13 (poly(methylmethacrylate-co-dimethylacrylamide)) and PA515 (poly(methoxyethyl)methacrylate-co-diethylaminoethylacrylate-co-methylmethacrylate)), were used to coat catheters, and substantially shown to decrease binding of a variety of bacteria (including isolates from infected endotracheal tubes and heart valves from intensive care unit patients). Catheters coated with PA13 showed up to 96% reduction in bacteria binding in comparison to uncoated catheters.
Trichomes are almost ubiquitous in the plant kingdom and are of significant commercial interest for the secondary metabolites secreted by glandular cells at the tip. For example, *Artemisia* produces the widely prescribed antimalarial drug Artemisinin, in glandular trichomes. Other plants such as tomato, produce chemicals which defend against pests and are therefore of value to the agricultural industry. Because the model plant *Arabidopsis* is incapable of producing such compounds, there is demand for a different model system for their study. The Asterid, *Antirrhinum*, is a strong candidate due to its species exhibiting diverse trichome phenotypes, ranging from simple unicellular trichomes to more complex multicellular structures with secretory capabilities. The *Hairy* gene is responsible for natural variation in the density of trichome production on vegetative tissues. It is thought that *Hairy* is a repressor of hair development because it segregates in a classic 3:1 (bald:hairy), Mendelian inheritance pattern. A variety of next-generation sequencing techniques have been employed to map *Hairy* and to identify transcripts associated with hairiness or baldness, with the intention of identifying *Hairy* and its role in multicellular trichome development.
Evidence indicates that the microorganisms that inhabit the intestinal tract play a role in shaping host bodyweight and metabolism. My research is using a mouse model to investigate how an obesity inducing high-fat diet causes changes in the composition of intestinal microbiota and how this can be linked to observed changes in the bodyweight of the mice. The hypothalamus is a key brain centre for the regulation of bodyweight and there are potential links between changes in the gut microbiota and changes in bodyweight regulatory system in this part of the brain. Previous work proposed that inflammation via the innate immune system was driving mechanisms linking changes in intestinal microbiota to obesity and that blocking this immune response in gene knockout mice could ameliorate the effects of an obesity inducing diet. Whilst investigating this further, my results show in contrast, that these gene knockout mice were actually not protected against obesity as proposed. Using denaturing gradient gel electrophoreses I am investigating what changes occurred in the microbiota in wild-type and the gene knockout mice on different diets. This will be followed by analysis of particular bacterial species responding to dietary and bodyweight changes. Using microarray analysis I am also looking at changes in gene expression in the hypothalamus of a subset of these mice to identify target genes for further investigation. The aims are to identify microbial changes in the mouse intestinal tract and to link these to neurological changes in the mouse brain potentially providing a mechanistic link between microbiota and obesity.
Prion diseases are a group of infectious neurodegenerative diseases found in both humans and animals. The infectious agent in these diseases is the prion, the exact physical nature of which remains to be confirmed. Despite this it has been widely accepted in prion research that a misfolded form of the endogenous prion protein (PrPsc) is the infectious agent. Although PrPsc is commonly found to accumulate in prion diseases it has been recently discovered that its accumulation in the form of amyloid plaques can occur in the absence of infectious prion disease.

This project aims to study the mechanisms of amyloid plaque formation in real time using brain organotypic slice culture (BOSC). LI-COR Odyssey and confocal imaging systems will be utilised to visualise plaque formation in BOSCs following exposure to recombinant PrP amyloid seeds.

To assess the effect of plaques on cell survival cell viability will also be tested. BOSC from wild type mice have been established and characterised in culture for 8 months. Cells of the BOSC from both wild type and transgenic mice have been investigated using a range of markers for viability, nuclei, stress, neurons and glia.
Barley is a key global cereal and an excellent genetic model crop for studying other temperate cereals such as wheat. The cereal stem, called a culm, is segmented into nodes and internodes. Culm internode length determines overall plant height, which is an important agronomic trait. The grain bearing spike at the tip of the stem (culm) is also separated into nodes and internodes. Our research has shown that microRNA172 (miR172) inhibition of its target HvAPETALA2 (HvAP2) underlies internode elongation in the barley culm and spike (Houston and McKim et al., 2013). Loss of miR172-regulation in the dense-spike, semi-dwarf barley mutant called Zeocriton (Zeo) was associated with overexpression of HvAP2, suggesting that HvAP2 is a repressor of internode growth. To begin to understand the mechanisms underlying HvAP2 repression of internode growth, we used fine-scale imaging techniques to compare cellular proliferation and elongation during internode development in wild-type and Zeo. Our results indicate that dwarfism in Zeo reflects both reduced cell number and cell elongation. We found that reduced cell number in Zeo results from dampened mitotic activity in the internode meristem, suggesting that HvAP2 suppresses meristematic cell proliferation. We also observed a previously undescribed hyperelongation event in specific cell populations at the base of the wild-type internode. Hyperelongation was absent in Zeo, also contributing to its dwarfism and indicating that miR172-regulation of HvAP2 is critical for this event to occur in wild type. To further establish the molecular mechanism of HvAP2 suppression of internode length, we are conducting comparative transcriptome analyses in proliferative and expanding zones within wild-type and Zeo internode tissue at different developmental stages. These data will highlight the regulatory transitions in gene expression along these zones within growing internodes and also identify potential targets of HvAP2 regulation.

References:

Our guts are home to trillions of commensal bacteria which live quite happily there causing us no harm whatsoever. The gut microbiota community has co-evolved with the host and can confer beneficial effects, such as metabolizing nutrients, modulating immune responses and assisting in defence against pathogens. The gastrointestinal tract is protected from these bacteria (or other damaging substances) by a layer of tightly packed epithelial cells which form a barrier against any bacteria or molecules penetrating the gut. However, Microfold (M) cells are specialised intestinal cells located over mucosal lymphoid tissue called Peyer's patches which are potential entry points into the host. M cells sample microorganisms or molecules in our gut and help transport them across the epithelial cell barrier (a process called antigen transcytosis) to deliver to professional immune cells (like macrophages, T cells or dendritic cells) to stimulate a protective immune response. In essence, M cells act like CCTV cameras to survey the gut area for anything that is out of the ordinary, or potentially harmful, and then present them to our immune system to sort those bad ‘uns out. There is potential for M cell manipulation to improve the efficacy of mucosal vaccines and help develop therapies to block orally transmitted diseases.
Two ‘alleles’ of the nonstructural (NS) segment circulate in non-chiropteran influenza A viruses. The ‘A-allele’ is found in avian- and mammalian-adapted viruses, but the ‘B-allele’ is almost exclusively avian. A plausible hypothesis for the restricted host range of the B-allele is that its NS1 protein is maladapted for controlling the mammalian immune response. To test this, a variety of avian influenza virus NS genes were reassorted into the mouse-adapted A/Puerto Rico/8/1934 (PR8) virus. All viruses replicated to similar titres in a variety of human and other mammalian cell lines, and also showed similar sensitivities to IFN-ß treatment. While in vitro co-infection (“competition”) studies revealed subtle fitness differences between WT PR8 and exemplar A- and B- allele reassortants, the order was PR8 > B > A. These viruses replicated to similar titres in the lungs of infected mice, and provoked indistinguishable lung pathology. However, both viruses with an avian segment 8 caused less weight-loss than parental PR8. Surprisingly, the A-allele virus showed the greatest attenuation. Both reassortant viruses elicited lower amounts of pro-inflammatory cytokines in the lung than WT PR8. Thus the B-allele of NS1 does not necessarily attenuate virus replication in a mammalian host but does attenuate disease.

**Keywords:**

Influenza, NS1, Allele, Reassortant, host range, attenuation, adaptation.
The endocannabinoid system (ECS) has classically been known as a major neuromodulatory system of the central nervous system in which endocannabinoids act as retrograde neurotransmitters that inhibit synaptic activity. However, recent evidence has suggested that the ECS has a key role in energy balance by acting at both central and peripheral target sites, which is of significance given that there is mounting evidence that suggests that the ECS becomes dysregulated during obesity.

Studies in our lab have demonstrated that manipulating the activity of cannabinoid receptors induces significant modulation of insulin sensitivity in tissues such as skeletal muscle. Thus, the aim of this project is to define the role that these receptors play as part of the peripheral endocannabinoid system with respect to metabolism and energy homeostasis in tissues such as skeletal muscle.

Preliminary results have indicated that incubation of cells with synthetic cannabinoid receptor 1 (CBR1) antagonists (Rimonabant and AM6545) promotes an increase in AMPK phosphorylation/activity, as judged by phosphorylation of its physiological downstream target ACC. Further work is needed to fully understand the mechanisms by which CBR1 antagonists induce increases in AMPK activity, however initial results indicate a potential role for calcium and activation of CAMKKβ, which functions as an upstream activating AMPK kinase. Given that AMPK plays a central role in energy sensing and a stimulant of oxidative fuel metabolism the results raise the possibility that CB1 receptor blockade may represent a potential strategy for treating metabolic dysfunction associated with obesity.
Matthew R. McFarland

Using Ribosomal feedback loops to control gene expression in synthetic biology circuitry

M. Carmen Romano, Ian Stansfield

Institute of Medical Sciences, University of Aberdeen

In eukaryotic cells, translation termination and release of a completed peptide is mediated by the eRF1/eRF3 protein complex. eRF3 brings eRF1 to the ribosome where it recognises the stop codon and, mediated by eRF3-driven GTP-hydrolysis, triggers release of the completed peptide. Following this, the ribosome is recycled by the eRF1 interacting factor Rli1. All eRF1 homologues contain a highly-conserved GGQ amino acid motif, believed to be the site of peptide release activity. Mutation of this universally conserved motif results in a dominant negative slow growth phenotype accompanied by stop codon readthrough. Stop codon readthrough is an example of a translational recoding event that results from the decoding of the stop codon by a sense tRNA allowing translation to bypass the stop signal.

In this work, we investigate the use of translational recoding signals to regulate gene expression in synthetic biology circuitry. We have C-terminally fused GFP to a tetR inhibitory peptide (TIP), separating the two domains with a translational recoding signal. The triggering of this event is controlled by the use of a tet-regulated tRNA expression system. This tRNA can affect the efficiency of TIP production, which inhibits tetR and influence tRNA expression setting up a feedback loop. By altering the translational recoding signal used, it will be possible to change the control properties of the system.

The results of this study aim to provide fresh insight into the competition between recoding signals and termination, as well as the use of these processes as a tool to regulate gene expression.
Alison McGarvey  Global gene expression analysis in elucidating the molecular characteristics of emerging hematopoietic stem cells

Dr Simon Tomlinson  University of Edinburgh

The recent development of high throughput next generation sequencing technologies has become a powerful method of investigating gene regulation in biological processes. One such process is the regulation of hematopoietic stem cell emergence. Hematopoietic stem cells (HSCs), the founders of the blood cell hierarchy, first appear in the developing mouse between embryonic day 10 and day 11. Whilst the timing and location of HSC ontogenesis is well described, the regulation of this event, via the signalling between precursor HSCs and their niche, is poorly characterised at a molecular level. By generating and analysing RNA sequencing data from the in vivo niche for HSC emergence we are investigating genes which potentially support HSC development. We have tested these in an ex-vivo culture system and have validated some genes which functionally support HSC development, suggesting that this is an effective method for characterising the niche. Through continued screening of candidate genes as well as more detailed mechanistic analysis of positive candidates, we aim to yield further molecular understanding of HSC development.
NFκB is a dimeric transcription factor that is important in inducing or repressing the expression levels of genes that have important implications in apoptosis, proliferation, inflammation, immune responses, angiogenesis and cancer and hence has important consequences in cell growth and survival. The specific genes that NFκB may activate depend upon the period of nuclear-cytoplasmic oscillations of NFκB protein, which in turn depends upon factors such as cell type, genetic background, stimulus type and stimulus exposure variability i.e. continuous or pulsatile [1]. The specific function of NFκB has been seen to be very diverse. If a cell has become cancerous or there is a dysfunction in the NFκB signalling pathway, then the function of NFκB can aid in tumour progression or tumourigenesis. However, depending on cell type, genetic background and stimulus, NFκB has been known to also act as a tumour suppressor. The NFκB pathway can also, unfortunately, be activated during chemotherapy to counteract the intended chemotherapeutic effects and once again aid in cell survival and hence tumour progression. Although, it has recently become apparent that the effects of cytotoxic drugs on NFκB function are also quite diverse and may differ between cell and tumour types or even the different stages of tumour development [2].

Due to the stochastic nature of intracellular signalling pathways and the compartmentalization of their occurrences in a cell (e.g. nucleus, cytoplasm), we present a spatial stochastic model of the NFκB signalling pathway within a single cell. Initially, a three-dimensional spatial stochastic analogue of the model in [3] is developed, where the two target genes of NFκB that act as the main sources of negative feedback are included. The model considers the reactions and diffusion of all the key molecular agents in the NFκB pathway and can predict the spatial distribution of proteins and mRNA molecules within a cell as well as the concentration profiles of the molecules over time (cf. [4]). An extension of the model considers the crosstalk with HIF1 and p53. We believe the analysis of the intracellular signalling pathways involving NFκB and the cross talk between them will lead to a greater insight into the complex roles these pathways play and ultimately into drug-targeting therapies. Better predictions of the effect of a drug or radiation treatment on NFκB at the tumour diagnosis stage might help match the type of therapy to the patient. Furthermore, such approaches might help predict when inhibiting NFκB in a tumour will also inhibit, rather than
stimulate, tumor growth. Overall, the diversity of NFκB functions should not be viewed as reasons for why therapies based on its function will not work. Rather, an increased understanding of the different roles and regulations of NFκB will create opportunities for the development of techniques that will allow existing and new therapies to be more effective. This knowledge could result in novel approaches to modulating NFκB function for the treatment of cancer and many other diseases.

References


Hannah Parkin  Analysis of heparan sulfate function in the developing mammalian forebrain using systematic gene expression analysis

Dr Tom Pratt  University of Edinburgh

Heparan sulfate proteoglycans (HSPGs) are a family of molecules involved in regulating key signalling events required for normal mammalian brain development. It is thought that specificity of HSPGs for particular signalling processes is encoded by the heparan sulfate (HS) sugar side chains, which can be modified post-synthetically to yield huge variation in HS structure. Different sulfation patterns are generated by the action of enzymes such as the heparan sulfate sulfotransferases (HSTs) and sulfatases. Depending on the expression of these enzymes and the resulting heparan sulfate ‘code’, it is proposed that cells are then able to regulate signals they receive and send in the ligand rich extracellular environment of the developing forebrain. Following loss of the two HSTs Hs6st1 or Hs2st that add sulphate groups to specific positions on residues of the HS side chains, commissural tracts including the corpus callosum fail to develop normally during late mouse embryogenesis. The telencephalic midline environment is perturbed, with a striking mis-positioning of glial cell populations that normally act to guide axons towards the contralateral hemisphere. One hypothesis to explain this phenotype is a change in critical cell populations and processes at a time when the correct midline environment is being established, that may be identified by changes in gene expression. Given the function of HS these changes might correlate with alterations in signalling such as an increase in Fgf/ERK that has already been found. We performed RNA sequencing analysis on dissected midline regions of WT, Hs2st−/− and Hs6st1−/− mouse embryos at E16.5 and have identified lists of differentially expressed genes. We find changes in gene expression in cell populations that are known to be important for corpus callosum development, including callosal pioneer axons and the disrupted glial cells observed in HST-null embryos. By comparing the expression of these genes between WT and mutant embryos we are gaining an insight into the underlying mechanisms of HS function.
Rhythmic behaviours such as breathing, chewing, walking and swimming are controlled by specific neuronal circuits in the spinal cord. The activity of these circuits is subject to modulation from descending inputs from the brain, which allows for behavioural flexibility. Dopamine (DA) is an important example of a neuromodulator, but compared to other amines little is known about the effects of DA on locomotor networks controlling swimming in amphibians. We looked at the effects of DA on the electrical properties of the neurons controlling swimming in young tadpoles, and how these cellular effects translate into modifications of behaviour.

We found that DA activates D2-like receptors in the membranes of neurons and this results in the opening of a potassium channel called a GIRK channel. This reduces the excitability of the spinal neuronal circuitry. At the behavioural level, DA caused a decrease in evoked swim episode duration and swim frequency, and an increase in the threshold for sensory stimulation of swimming. These purely inhibitory effects were mimicked by quinpirole, an agonist for the D2-like receptors, whereas raclopride, a D2-like antagonist, had the reverse effects. Interestingly, blockade of D2-like receptors also induced spontaneous swimming activity in the normally silent network, suggesting that in early larval development spontaneous rhythm generation is suppressed in the spinal cord by tonic DA-mediated inhibition.

Finally, we found that during development the inhibitory effects of DA described above switch to excitatory during larval development.
Path integration is a basic navigational strategy observed across a wide range of species whereby an animal estimates its current position relative to a reference point. This requires that the animal continually infers its direction and distance moved according to idiothetic, or internally generated, cues. While there is good evidence that animals can infer their position by path integration, how the brain combines sensory signals to do this is unknown. Grid cells in layer II of the medial entorhinal cortex fire action potentials at locations that form a striking grid-like pattern that tiles the environment. Several properties of grid cells indicate their involvement in path integration, including their co-localization with head direction and conjunctive cells that exhibit spatially tuned firing; so all the information required for path integration is present in the local circuit. This has lead to the prediction that grid cells are the path integrator. We tested this prediction by specifically blocking the synaptic output of grid cells in mice, and then training them to locate a reward zone in a virtual linear track. In some trials, the reward zone was marked with a beaconing cue whereas in others it was invisible. In beaconed and non-beaconed trials both control mice and mice with inactivated grid cells were able to consistently locate the reward zone, but they did this using different strategies. The control mice stopped preferentially in the reward zone whereas mice with inactivated grid cells stopped indiscriminately. This suggests that inactivation of grid cells impairs both beaconing and path integration strategies of navigation.
Rebekah Tillotson Are the abilities to bind methylated DNA and the NCoR/SMRT repressor complex sufficient for MeCP2 function?

Professor Adrian Bird University of Edinburgh

Every cell in our bodies contains the same set of instructions, our DNA. However, in different cell types, different genes must be switched on and off. Modifications to the DNA and its packaging proteins help regulate whether genes are activated or silenced. DNA methylation is the addition of a methyl group to cytosine bases of DNA. This mark can prevent the genes being switched on by disrupting the binding of activator proteins; or by recruiting repressor proteins that manipulate the structure of the packaging proteins, making the DNA inaccessible. MeCP2 is a protein that binds methylated DNA and is highly expressed in neurons. Loss-of-function mutations in the MeCP2 gene cause Rett Syndrome, an autism spectrum disorder that affects 1:10,000 girls. These mutations exist almost entirely in the regions of MeCP2 that bind methylated DNA and to a complex of repressor proteins, called NCoR/SMRT. This suggests that MeCP2 may function as a bridge between methylated DNA and the NCoR/SMRT complex. If this hypothesis is true, deleting the uncharacterised regions of MeCP2 will not affect protein function. I will test this hypothesis by manipulating the MeCP2 gene in mouse models, and then carry out behavioural tests on these knock-in mice to determine whether they are healthy or exhibit symptoms of Rett Syndrome. This study will help further our understanding of the mechanisms of gene regulation.
Alternative splicing (AS) regulates gene expression in the Arabidopsis circadian clock at the post-transcriptional level. Splicing factors (SFs) are trans-acting proteins that interact with cis elements to regulate the accurate splice site selection of variable downstream genes under different environmental conditions. Experimental data show that many splicing regulators/RNA-binding proteins (RBPs) are alternatively spliced and this most likely represents a mechanism of regulating splicing factor levels. Changes in the AS patterns of SFs affect their expression levels and abundance and as a consequence the AS of downstream regulated genes. Low temperature induces specific changes in AS patterns of core clock genes (e.g. LHY, PRR7). Although extensive post-transcriptional regulation of gene expression of the core clock genes in plants by AS has been described, the underlying mechanisms and factors that control stress-sensitive and more specifically cold-sensitive AS are unknown. The aim of my PhD project is to study the effect of low temperatures on the AS and expression patterns of Arabidopsis circadian clock-associated genes and regulatory splicing factor genes, and to identify SFs which are involved in the regulation of temperature-dependent AS of clock genes. To address our main scientific objective two different approaches are being taken: a candidate gene approach and a RNA-seq experiment. So far 56 RBPs have been identified with rhythmic and cold-responsive expression, many with significant AS. Further investigation of the genes is carried out in parallel with the data from the RNA-seq experiment for the identification of major RBPs/SFs regulating cold-induced AS in the circadian clock.
A group of chemotherapeutic drugs known as anti-mitotics act to prevent cell proliferation by arresting cells in mitosis. These drugs work by inhibiting microtubule polymerisation or depolymerisation resulting in several different fates. One possible outcome is cell death through the intrinsic apoptotic pathway which can be initiated by cell stress and is controlled by both pro-apoptotic and pro-survival proteins. The pro-apoptotic proteins function by neutralising pro-survival proteins; this allows release and activation of proteins called Bax and Bak which insert into the mitochondrial membrane forming pores. Cytochrome c is then released from the mitochondria resulting in caspase activation and subsequent apoptosis. The control of apoptosis in mitosis is not fully understood but it is known to require a fine balance between pro-apoptotic and pro-survival proteins. The aim of this project is to explore the pro-apoptotic role of the proteins Bax and Bak in response to mitotic arrest. To do this, cells were treated with the drug nocodazole, which depolymerises microtubules and causes cells to arrest in mitosis. siRNA was used to knockdown Bax or Bak proteins and the effects on cell death in response to mitotic arrest were assessed. An increased understanding of the role of Bax and Bak in cell death during mitosis is crucial to exploit the intrinsic apoptotic pathway in current and future cancer therapies.
# Part II. Poster session by EASTBIO first-year students, by theme

## Poster session for Basic Bioscience underpinning Health (Ageing)

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Student’s PhD Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curley, Michael</td>
<td>Establishing the cause-consequence relationship between the age-related reduction in androgens and normal ageing process</td>
</tr>
<tr>
<td>Graham, Laura</td>
<td>Mitochondria and synaptic stability</td>
</tr>
<tr>
<td>Green, Cara</td>
<td>Metabolomic analysis of responses to caloric restriction</td>
</tr>
<tr>
<td>Henderson (nee Walker), Audrey</td>
<td>Skin carotenoids and immune function</td>
</tr>
<tr>
<td>Hope, Jilly</td>
<td>The role of eEF1A2 in epilepsy, intellectual disability and autism</td>
</tr>
<tr>
<td>McGregor, Gemma</td>
<td>Age-dependent effects of leptin on hippocampal excitatory synaptic plasticity</td>
</tr>
<tr>
<td>Moatt, Joshua</td>
<td>Meta-analytical insights into the effect of dietary restriction on reproductive investment</td>
</tr>
<tr>
<td>Tsang, Hiu-Gwen</td>
<td>Exploring regional expression of calcification genes in the sheep cardiovascular system</td>
</tr>
</tbody>
</table>

## Poster session for Bioenergy & Industrial Biotechnology

<table>
<thead>
<tr>
<th>Student’s name</th>
<th>Student’s PhD Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewster, Richard</td>
<td>Biotinidase Resistant Biotin-DOTA conjugates: Synthesis and applications in Imaging</td>
</tr>
<tr>
<td>Doble, Megan</td>
<td>Artificial Metalloenzymes as Catalysts for Oxidative Lignin Degradation</td>
</tr>
<tr>
<td>Mittmann, Sybille</td>
<td>The control of meiosis and recombination in barley</td>
</tr>
<tr>
<td>Pietrzyk, Julian</td>
<td>Use of Microbial Consortia for Conversion of Biomass Pyrolysis Liquids into Value-Added Products</td>
</tr>
<tr>
<td>Tuck, Laura</td>
<td>Engineering Bacterial Microcompartments as platforms for synthetic biology</td>
</tr>
<tr>
<td>Author</td>
<td>Title</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Aleksandrova, Yana</td>
<td>The neuroendocrine and genetic control of maternal behaviour in the domestic hen</td>
</tr>
<tr>
<td>Dehler, Carola</td>
<td>Gut health and nutrition in Atlantic salmon (<em>Salmo salar</em>)</td>
</tr>
<tr>
<td>Draicchio, Fulvia</td>
<td>Using a barley Nested Association Mapping Population to mine wild barley germplasm for crop improvement</td>
</tr>
<tr>
<td>Gavan, Martha</td>
<td>Gene dynamics of Toll-like receptor 4 through a population bottleneck in an insular population of water voles (<em>Arvicola amphibious</em>)</td>
</tr>
<tr>
<td>Glaser, Georgina</td>
<td>Context-dependent decision making in rufous hummingbirds (<em>Selasphorus rufus</em>) and parasitic wasps (<em>Nasonia vitripennis</em>)</td>
</tr>
<tr>
<td>Harbottle, Jennifer</td>
<td>The environmental stress response as a target for therapeutic intervention</td>
</tr>
<tr>
<td>Heath, Sarah</td>
<td>Studying fitness in the unicellular marine alga <em>Ostreococcus tauri</em></td>
</tr>
<tr>
<td>Jäger, Franziska</td>
<td>The ESX-1 protein secretion system in Bovine pathogen <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Johnston, Christopher</td>
<td>The Mechanism and Functional Significance of Spliced Leader Trans-Splicing in Nematodes</td>
</tr>
<tr>
<td>Mathie, Heather</td>
<td>Defining Mycobacteria Specific Responses in Macrophages: Towards Identification of Targeted Approaches for Disease Control</td>
</tr>
<tr>
<td>Moseley, Mark</td>
<td>The genetic diversity of <em>Leptospira</em> in Madagascar</td>
</tr>
<tr>
<td>Murphy, Fraser</td>
<td>Characterisation of a host MAP3-Kinase targeted by an effector protein from the late blight pathogen, <em>Phytophthora infestans</em></td>
</tr>
<tr>
<td>Pyott, Douglas</td>
<td>Effects of light and temperature on Potyvirus infection</td>
</tr>
<tr>
<td>Regan, Charlotte</td>
<td>Home range quality and the cost of reproduction in female Soay sheep (<em>Ovis aries</em>)</td>
</tr>
<tr>
<td>Reid, Angus</td>
<td>Understanding how domestication has altered growth utilising chicken lines segregating for a mutation affecting satiety</td>
</tr>
</tbody>
</table>
## Poster session for World Class Bioscience

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashton, Anna</td>
<td>A new function for the Pineal Gland and control of nightly melatonin rhythm</td>
</tr>
<tr>
<td>Bertolini, Simone</td>
<td>Defining mutational signatures from DNA damage processes by exploiting Caenorhabditis Elegans</td>
</tr>
<tr>
<td>Bisset, Emma</td>
<td>Plant root associated <em>Bacillus subtilis</em> biofilm formation</td>
</tr>
<tr>
<td>Brodaczewska, Natalia</td>
<td>Production of L- and P-selectin protein constructs for structural NMR studies using <em>P. pastoris</em> expression system</td>
</tr>
<tr>
<td>Chapman, Elliott</td>
<td>Investigating the mechanism and function of RNAi across fission yeast species</td>
</tr>
<tr>
<td>Dicks, Kara</td>
<td>Unravelling the disease and fitness consequences of MHC diversity in a uniquely well-characterised sheep population</td>
</tr>
<tr>
<td>Glendinning, Laura</td>
<td>The Lung Microbiota</td>
</tr>
<tr>
<td>Kaemena, Daniel</td>
<td>CRISPR/Cas9 Genome-wide screening to investigate the molecular mechanisms of reprogramming</td>
</tr>
<tr>
<td>Wheeler, Emily</td>
<td>Investigating targets of the RNA methylation complex and their interaction with the flowering promoter FPA</td>
</tr>
</tbody>
</table>
Androgens (e.g. testosterone and dihydrotestosterone), produced by testicular Leydig cells, are essential for complete masculinisation/reproductive tract development during fetal life and maintenance of reproductive function/secondary sex characteristics in adulthood. Longitudinal studies show that as males age, androgen levels decrease. Recent studies suggest that androgens may also be important for maintenance of general health in adult males, with low androgen levels inversely associated with cardio-metabolic disease risk. However, the cross-sectional nature of such studies restricts any interpretation of causality (i.e. whether low androgen levels drive disease progression or vice versa). Considering the increasing proportion of older males in developed countries, it is essential the relationship between ageing, androgen levels and cardio-metabolic diseases be further explored.

Central to this is a well-developed understanding of the mechanism(s) that regulate adult LC function and the mechanism(s) by which LC function deteriorates with age. Such studies may identify putative therapeutic targets and/or novel ways to promote healthy ageing in males thus reducing the healthcare burden of an increasingly older population. In mice, Cisd2 expression decreases with age and Cisd2−/− animals show early onset of age-associated phenotypes. We aim to generate a novel mouse model in which premature ageing (Cisd2 deletion) is restricted to LCs. To this end, we assessed the utility of a Pdgfrb-Cre mouse for LC specific Cisd2 deletion using a RFP reporter line. We found that Pdgfrb-Cre is expressed by stem/progenitor LCs in the fetal testis and targets a proportion of LCs in adulthood.
Mitochondria are the ‘power-houses’ of all cells, generating ATP to fuel numerous pathways which are vital for cellular form and function [1]. Neuronal processes and synapses present a constant demand for ATP to maintain ionic gradients and neurotransmission events [2], promoting sub-populations of mitochondria to be enriched pre- and post-synaptically [3, 4]. These mitochondria display unique enzymatic [5], calcium buffering [6, 7] and antioxidant properties [8] and have thus been associated in the pathogenesis of a variety of neurodegenerative diseases where the synapse is the primary target. We have characterised the proteomes of these synaptic and non-synaptic mitochondria at a basal level by following early biochemical isolation methods [5] and adopting a label-free proteomic approach, generating a species-specific molecular fingerprint. Our study has demonstrated distinct proteomic profiles between the two sub-populations of mitochondria, dependent upon sub-cellular localisation. We have used quantitative fluorescent western blotting to validate the proteomic studies in a range of species, suggesting that the proteomic data may be an accurate reflection of the fingerprint for distinct mitochondrial populations. These results also suggest that mitochondrial neuronal sub-populations and their relative protein abundances are likely conserved between mammalian species.

References:


[6] Brown MR, Sullivan PG, Geddes JW. Synaptic mitochondria are more susceptible to 

permeability transition among brain regions in the rat correlate with selective vulnerability. *J 

Cara Green  Metabolic analysis of responses to caloric restriction

Prof. John Speakman, Dr Alex Douglas & Dr David Lusseau
Aberdeen University

Caloric restriction has been practiced for over 500 years as a method of improving quality and length of life. The first quantitative experiments emerged in the early 20th Century and since then, positive effects of caloric restriction without malnutrition have been seen in a wide array of taxa including Drosophila, Caenorhabditis elegans, fish and mammals. Most notably, rodent lifespan can extend by up to 50% under caloric restriction. Caloric restriction not only increases longevity in rodents but also delays onset of many age-related diseases such as cancer and neurodegenerative disorders.

I aim to determine the metabolic mechanisms through which caloric restriction affects health. To do this I focus on how different degrees of caloric restriction (restricting the diet by 10%, 20%, 30% and 40%) affect the metabolome of mice. The metabolome is a key area of study as several metabolic pathways have been implicated in the positive effects of caloric restriction such as the insulin/IGF 1 and sirtuin pathways.

The hypothesis underlying my work states that, as an increasing level of restriction causes a more marked impact on lifespan, important factors underpinning this effect with also change linearly with the degree of restriction. I have shown that the number of significantly differentially expressed metabolites in the liver of C57BL/6 mice rises with increasing levels of caloric restriction after 3 months on a restricted diet. Additionally, my results indicate that fatty acid metabolism and signalling, GABA receptor, and nerve growth factor signalling pathways are most significantly altered in the liver after 3 months of restriction.
Fruit and vegetable consumption is known to increase the yellow tone of human skin (Stephen et al, 2011; Whitehead et al, 2012). Furthermore, this increase is perceived as more healthy and attractive (Whitehead et al, 2012), possibly because it signals the absence of need for carotenoids to act as antioxidants (von Schantz et al, 1999). Other lifestyle factors related to health are known to impact dermal levels of carotenoids (Darvin et al, 2008), but whether skin colour reflects overall health status, in addition to carotenoid consumption is unconfirmed.

Using spectrophotometry methods, we investigated the independent contributions of fruit and vegetable consumption (controlling for body weight), adiposity, and frequency of exercise upon skin yellowness (CIE b*), in a sample of 100 Caucasian participants. A bootstrapped linear regression confirmed that all three variables of interest were independent predictors. Exercise and fruit and vegetable consumption predicted higher b* values whilst body fat mass was associated with lower b* values. Furthermore we found that the strength of relationship between each predictor and dermal spectral reflectance varies in accordance with the known absorption profile of carotenoids, between 400 and 540nm.

Results suggest that carotenoid colouration of human skin reflects health related lifestyle factors beyond consumption of fruit and vegetables. Because carotenoid colouration of human skin is perceived as attractive, these findings provide further incentive to improve overall health by reducing excess weight, exercising regularly and adopting a diet high in fruit and vegetables.

References:


Jilly Hope

The role of eEF1A2 in epilepsy, intellectual disability and autism

Cathy Abbott and Mandy Jackson  The University of Edinburgh

Intellectual disability, epilepsy and autism are largely distinctive neurological disorders for which there is a major lack of effective treatments. Here, we have a mutation in a translation elongation factor gene, eEF1A2, through which patients present with all three disorders, providing an excellent opportunity to study the underlying mechanisms. In my project, I aim to establish the role of eEF1A2 in intellectual disability, epilepsy and autism by determining the effects of mutant eEF1A2 on neuronal cells, studying the potential interaction of eEF1A2 with other epilepsy proteins and assaying for behavioural phenotypes in mice which are consistent with those seen in these disorders.
Leptin is a neuroendocrine hormone encoded by the obese (ob) gene which is released predominantly from white adipose tissue and circulates in proportion to body fat content. Extensive study has indicated that leptin regulates energy homeostasis and acts as a satiety signal to regulate food intake however recent studies have implied that the hormone has a role in cognitive function. Human and rodent studies of obesity and Type II diabetes have highlighted a reduction in leptin signal transduction and subsequent cognitive impairment. Furthermore, a reduction in leptin correlates with ageing as well as an increase in Aβ, a pathological hallmark thought to be related to Alzheimer's disease.

The aim of this project is to examine the age-dependent effects of leptin on excitatory synaptic transmission and plasticity in the hippocampal rodent model and how alterations in the leptin system early in development impact excitatory synaptic function in the adult brain and with ageing. This investigation will focus on the impact of leptin at the perforant path input to CA1 synapses, a pathway in the hippocampus which displays the earliest signs of neurodegeneration in Alzheimer's disease and is thought to be specifically linked to the formation of episodic memory.
Dietary restriction (DR) has been at the forefront of ageing research since its conception in 1935. A restriction in intake of calories and specific macronutrients has been shown to increase lifespan and health-span among a plethora of other benefits. The evolutionary mechanisms behind the life extending effect are explained using the disposable soma theory. This theory postulates a trade-off between investment in somatic maintenance and reproduction. Under DR, when resources are limited, organisms shift resources away from reproduction towards somatic maintenance to improve the chance of survival until nutritional conditions improve and reproduction can recommence. This increase in somatic maintenance is thought to be the cause of the increase in longevity. Evidence for this trade-off is convincing in model organisms, however the case for non-model species is much less certain. To assess the universality of this trade-off we carried out a meta-analysis of the effect of DR on reproduction utilizing 21 species and 222 effect sizes. Our results show that in general DR does lead to a reduction in reproduction. However, this effect is much stronger in model organisms. Our results also indicate that there are striking sex differences in the effect, with females suffering a much stronger reduction in reproduction than males. However, we suggest this result may be due to very few studies allowing males to experience the most costly aspects of reproduction, such as courtship and male-male competition. Finally, our results indicate that the effect of DR is dependent on the type of reproductive trait being examined.
The transcriptional landscape of an organ or system is pivotal to its function. The cardiovascular system is key to maintaining health, and abnormalities of this system are a major cause of disease. However, there has been little research on gene expression in the components of this system. Thus, the aim of this project is to investigate the mammalian cardiovascular transcriptome, especially concerning genes involved in the pathological process of vascular calcification. Vascular calcification is prevalent in aging, hypertension and atherosclerosis, and is an independent, strong predictor of risk of cardiovascular-related death. Initially, quantitative real-time PCR has been performed for various genes involved in providing strength and elasticity to the cardiovascular tissues, and genes that influence calcification. This preliminary survey will be extended using genome-wide approaches. In this project, qPCR and RNA-Seq will be employed to measure gene expression levels from an array of cardiovascular tissues of a large animal model – the sheep. The innovative tool BioLayout Express 3D will be used to identify networks of co-expressed genes. Preliminary results from a healthy elderly female sheep indicate variable expression levels in fibrillin-1, fibrillin-2, ectonucleotide pyrophosphatase/phosphodiesterase 1, and the master osteoblast transcription factor, Runt-related transcription factor-2, throughout different cardiovascular regions. Interestingly, these genes exhibited higher expression levels in aortic tissue compared to other samples. With the goal of discerning the key underlying factors of cardiovascular risk, creating a genome-wide transcriptomic atlas of the mammalian cardiovascular system will provide further insights into differential expression patterns that contribute to region-specific functions.
Biotin is widely used in biological research, however one major drawback in these applications is provided by the enzyme biotinidase (BTD) found in serum. Whilst this enzyme is essential for managing biotin levels – it also causes rapid hydrolysis of amide and ester bonds of biotinylated reagents.

Current routes to conferring BTD resistance, such as reduction of the amide bond in ST2210, are effective but reduce binding to StAv by up to $10^5$ M. Sharpless highlighted the use of a triazole as an amide bond isostere and initial results from the Hulme group show that this substitution gives compound 1 that is BTD resistant and has binding affinity equivalent to the amide. We are synthesising a library of triazoles to determine how both triazole positioning and orientation affect StAv binding. Coupling of these BTD resistant compounds to Gd-DOTA gives complexes which can be used as contrast agents in MRI. We will optimise a pre-targeting method to investigate inflammation by imaging VCAM-1 expression in vivo.
In collaboration with industry we are also investigating the application of a BTD resistant version of the popular protein biotinylation product Sulfo-NHS-LC-Biotin.

*T1-weighted scan of 7 Eppendorf vials.*

*From top: 1) control; Dotarem® (250 mM); 2) triazole-linked biotin-Gd-DOTA complex 1 (500 mM); 3) (empty); 4) amide-linked biotin-Gd-DOTA complex (500 mM); 5) Dotarem® (500 mM); 6) vehicle, 7) water*

Lignin is an aromatic non-ordered polymer that gives plants their structural integrity by acting as a resin. The pulp and paper industry produces large quantities of extracted lignin and only a small percentage is used commercially and the rest is simply burnt as low value fuel. Where biorefineries focus mainly on the production of aliphatic platform chemicals from (hemi)cellulose, the degradation of lignin could provide a renewable source of low molecular weight aromatic compounds. 1

Artificial metalloenzymes are an elegant approach to combining both enzymatic and chemical methods to create the ideal solution. 2 Artificial Metalloenzymes combine the molecular recognition properties of enzymes, which are responsible for the high selectivities, with synthetic transition metal cofactors, to improve properties over the natural enzyme. This projects aim is applying artificial metalloenzyme systems to lignin oxidation for the sustainable production of high value added chemicals.

![Covalent modification of protein using maleimide ligand](image)

**Figure 1 : Covalent modification of protein using maleimide ligand**

Within the group we have created a library of nitrogen rich ligands for catalytic studies with a variety of metals. These ligands all contain a maleimide moiety that can use be utilised for covalent attachment to a free thiol group within a cysteine residue (Fig 1). The vast complexity of lignin has encouraged the use of simpler model compounds (Fig 2) to study lignins reactivity and can therefore develop catalysts which target specific linkages. Our results to date on the preparation of
artificial metalloenzymes will be presented alongside the application in benchmark catalysis reactions including the oxidation of lignin model compounds.

Figure 2: lignin model compounds β-O-4 linkage seen within lignin

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Economically important cereals, such as barley and wheat, have been shown to have skewed distribution of meiotic crossovers towards the telomeric ends of the chromosomes. This has potentially a negative impact on breeding efforts because natural variation in these genes cannot be easily accessed by traditional breeding methods. Moreover it reduces the efficiency of selection as disadvantageous linkages (e.g. between good and bad alleles) cannot easily be broken. In order to better understand this skewed distribution of crossing over and gain more knowledge about meiosis and recombination in cereals crops, I am fine mapping and characterizing the barley desynaptic mutant des12 in order to test whether manipulating des12 can alter the pattern of recombination. des12 is a spontaneous mutation, which was found in the cultivars ‘Betzes’ and ‘Freja’ and has two known mutant alleles. Phenotypically des12 mutants are severely semi-sterile and are characterized by an abnormal synaptonemal complex during prophase I leading to improper chromosome segregation due to missing ‘linking’ chiasmata, which are the physical manifestations of crossovers. Near-isogenic lines (BW233 and BW232) were created by backcrossing these alleles with the common cultivar Bowman (wt). Using a large F2 population derived from a cross between BW233 and Barke I have fine mapped des12 to a sub-centiMorgan region on chromosome 7H. The mapping interval contains about 19 genes based on the synteny to rice and Brachypodium. Initial data suggests that the gene is not a known meiotic gene and has not been characterized in any species including the model plant Arabidopsis thaliana.
An ever increasing demand for renewable fuels and chemicals has highlighted lignocellulosic biomass as a major source of available carbon; however the recalcitrant nature of lignocellulose makes it a particularly difficult compound to degrade. Pyrolysis is able to thermally depolymerise the constituent compounds of biomass making them easier to extract which can subsequently be done utilising consortia of microorganisms which can further degrade and create potential value-added products. The aim of this study is to monitor changes in pyrolysis oil as natural and synthetically augmented microbial communities degrade the biomass and generate a value-added product.

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Laura Tuck Engineering Bacterial Microcompartments as platforms for synthetic biology

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Bacterial Microcompartments (BMCs) are proteinaceous structures that occur in a wide variety of different bacteria, and compartmentalize metabolic processes within bacteria that produce volatile or toxic intermediates. The most well characterized BMC is the carboxysome, which houses the Rubisco and carbonic anhydrase enzymes that are used in carbon fixation to increase this processes’ efficiency. More recently discovered BMC’s include the Eut microcompartment, which was first characterized in Salmonella and has also been discovered in Clostridium difficile. The Eut locus encodes various different genes for microcompartment shell proteins, as well as enzymes that are involved in the ethanolamine breakdown pathway. Clostridium phytofermentans is a Gram-positive bacteria in which a BMC for the breakdown of fucose and rhamnose in the 1,2-propanediol pathway has been suggested.

The aims of this project are to first clone the fucose-rhamnose BMC shell proteins in C. phytofermentans and to find whether any of these proteins bind to the aldehyde dehydrogenase (Cphy_1178) in this pathway, to help to better understand how enzymes are recruited into the BMC. The understanding of enzyme recruitment will hopefully lead to the ability to direct enzyme pathways into synthetic compartments. The project also aims to produce a synthetic vector system for screening a wide range of potential localization sequences to investigate their binding properties to shell proteins. The ultimate aim of the project is to build fully synthetic compartments, and to add a novel enzyme pathways into them, as proof of concept that microcompartments can be used in a broad range of applications.
Yana Aleksandrova

The neuroendocrine and genetic control of maternal behaviour in the domestic hen

Dr Simone Meddle and Dr Ian Dunn
University of Edinburgh

The neuropeptides oxytocin and vasopressin have been found to play a crucial role in social behaviour in mammals, including maternal behaviour. The avian orthologues – mesotocin and vasotocin – are closely related peptides (differing in one amino acid), which suggests that these peptides may fulfil a similar role in birds. Prolactin, (a hormone secreted from the anterior pituitary gland), together with its releasing factor vasoactive intestinal peptide (VIP), has also been implicated in parental behaviour in a diversity of species, including the chicken.

Evidence in the literature suggests that the mechanisms for control of many social behaviours including parental behaviour have been highly conserved throughout evolution. Recent findings in the chicken and turkey strongly suggest that mesotocin may be necessary for the rearing of chicks after hatching but the exact mechanism has not been discovered and, to our knowledge, the involvement of vasotocin in avian maternal behaviour has not been investigated.

This PhD project will test the hypothesis that vasotocin and mesotocin are involved in the control of maternal behaviour in the domestic hen, from incubating right through to chick rearing, and investigate the possible mechanisms of their action. In the case of mesotocin, a sequence of related events involving prolactin and the dopaminergic system (mainly through the D2 dopamine receptor) can be inferred from the existing literature. The validity of this proposed mechanism will be examined in the course of our research.
One of the biggest challenges in aquaculture of Atlantic salmon (*Salmo salar*) is to provide the carnivorous fish with high quality diets required for good growth and health. Commercial feed for Atlantic salmon traditionally contains fishmeal and fish oil. However, this is not sustainable, as the small pelagic feed fish fishery is nearing over-exploitation. Alternatively, plant based proteins and oils have been proposed and implemented to certain inclusion levels. This often has adverse effects on the fish, as the organism has not evolved to cope with the relatively low nutritional value of plants. Although studies have been undertaken to investigate the influence of different diets on growth of the fish, little is known how those alternative diets influence the structure of the intestine, gut transcriptomics and the microbiota associated with a healthy intestine. Studies of gut microbiota have become increasingly important for dietary induced health issues in humans, for example inflammatory bowel disease. Those studies rely mainly on the 16S rDNA target to identify the different microbes in the gut and pin down the main players. In fish, this technology has only been implemented recently and little information is known about what a “healthy” gut microbiota is and how susceptible it is to altered diets and disease outbreaks. To investigate the microbiota of fish in facilities fed commercial diets, fresh- and seawater samples will be collected. Microbial 16 S rRNA sequences will by obtained by next generation sequencing. This will form the starting point for further investigations into gut health.
Cultivated Barley (*Hordeum vulgare* spp. *vulgare*) is the fourth most important cereal worldwide. However, its domestication led to a dramatic loss of allelic diversity. A possible strategy to recover this lost biodiversity is adding exotic alleles from wild species in breeding programs, since exotic germplasm is a source of allelic diversity. However, some problems are linked to germplasm use, such as lodging, seed dispersal as well as difficulty in finding accurate genetic and genomic locations of beneficial quantitative trait loci (QTLs). Moreover, the common mapping strategies to find beneficial QTLs have several limitations: linkage analysis method has a poor resolution in QTL detection, whereas association mapping analysis presents low power in detecting QTLs.

To overcome all these issues, I am using a Nested Association Mapping (NAM) population called HEB-25, which presents several advantages compared to the other methods: ultra-high mapping resolution, low marker density requirement, high statistical power and allele richness. My project involves two approaches: 1) Genome-Wide Association Scanning (SWAS) of the HEB-25 NAM Population, to discover gene alleles from the wild parents that encode beneficial traits, and 2) Generation of peri-centromeric (PC) substitution lines, to test the hypothesis that pericentromeric genome regions derived from the wild parent lines can contribute to useful gene alleles for crop improvement. Therefore, the SWAS analysis involves a) the assessment of allele content of all the HEB-25 lines, subsequent SNP discovery and SNP calling, b) simultaneous study of the HEB phenotypic performance in replicated field trials, and c) combination of genotype and phenotype data to identify wild barley alleles linked to improved plant performance under stress. Then, the HEB-25 population will be subject to exome capture and next generation sequencing for SNP calling and downstream association analysis.

In regard to the PC substitution line generation, a subgroup of HEB-25 lines are subject to backcrossing and further self-crossings to clean up the genome from undesired exotic segments which don’t cover the heterochromatic regions of HEB-25 genome. Since the PC heterochromatic regions are an important contributor to the narrow gene diversity in cultivated barley, their substitution with wild segments that are rich in gene diversity could dramatically increase the biodiversity of this crop.
Understanding how standing genetic variation at immunologically important genes varies in natural populations in response to demographic and population dynamic perturbation is essential for assessing the susceptibility of host populations to pathogens and parasites, and from that predicting the emergence and spread of zoonotic diseases that can affect livestock and wildlife populations.

Here, we focus on levels of allelic diversity and heterozygosity at the Toll-like receptor 4 (Tlr4) locus, a key gene involved in innate immunity. Variation is characterised across 280 water voles (Arvicola amphibious) from an isolated, island population located in north west Scotland that went through a severe bottleneck between 2004 and 2006, where genetic diversity is predicted to have been lost through the effects of genetic drift. Contrary to expectations, two functional alleles were retained at Tlr4 through the bottleneck, and an excess of heterozygote genotypes relative to Hardy-Weinberg expectations was observed before the population bottleneck. One of the Tlr4 alleles explained a significant proportion of the variance of flea (M. walkeri) and tick (I. ricinus) larvae burdens among individuals, suggesting a mechanism through which parasite mediated selection can affect TLR diversity.
Humans and other animals do not always make rational decisions. Instead, an individual’s decisions may be context-dependent, where decisions are affected by certain cues, such as the range of options present at the time of choice, or previous experience of options. The effects of these contexts on energetically costly decisions like egg laying (oviposition) in invertebrates such as parasitic wasps (Nasonia vitripennis) are largely unknown. We aimed to determine whether female Nasonia make context-dependent oviposition decisions by assessing whether decisions were affected by 1) presence of poor quality options in a choice set, 2) previous experience of options differing in quality. Female Nasonia parasitise blowfly pupae, but willingness to oviposit decreases if a host is already parasitised by another female. By allowing females to pre-parasitise hosts before focal females, we could manipulate host quality (high, medium, low, or very low). For experiment 1 we introduced a low or very low quality host into a choice set of a high and medium quality host, whilst in experiment 2 we gave females a host of a certain quality, then after 24 hours replaced the host with a high and medium quality host. We found that previous experience of a host affected Nasonia oviposition decisions, where willingness to lay on a medium quality host increased with decreasing quality of the host from the pre-experiment treatment. However, decisions were not affected by the presence of poor quality options in experiment 1. These results suggest that female Nasonia make context-dependent oviposition decisions.
Caloric restriction, post-natal growth restriction and genetic manipulation of the GH/IGF-1 axis extend lifespan, improve glycaemic control and protect against tumour formation in a wide range of species. Cells derived from long-lived animal models also show increased resistance to cellular toxins. Activation of the environmental stress response (ESR), driven by the transcription factor Nrf2, contributes towards these health benefits and it is therefore of interest to identify compounds that mimic these effects. Phytochemicals which stimulate the ESR would allow the development of novel therapeutic interventions which could stimulate endogenous cytoprotective mechanisms, thereby delaying the onset of age-related disease, extending health span, and promoting healthy ageing. In order to identify compounds that activate the Nrf2 pathway, a cell-based reporter system was established in human hepatocellular carcinoma HepG2 cells using a luciferase reporter gene under the control of the human NQO1 promoter. Sulforaphane, a potent isothiocyanate found in cruciferous vegetables that enhances Nrf2-regulated gene induction and up-regulates phase II detoxification enzyme production, was used to validate the reporter system. Responsive cell clones were selected and these cell lines were subsequently used to screen a natural product library from the Marine Biodiscovery Centre (University of Aberdeen). Several compounds elicited a potent response in luciferase activity, thus were further analysed to investigate endogenous gene expression and the mechanisms underlying Nrf2-driven chemical response.

**Keywords:**
Cell-based assay system, environmental cell stress response, aging, Nrf2.
The unicellular green alga *Ostreococcus tauri* is the smallest known eukaryote. It is widely used a model organism for green plants and algae because it has a very primitive and simple structure and it has had its genome sequenced. The control of cellular levels of proteins enables organisms to respond to environmental change. Proteomic analysis was used to examine various factors influencing fitness in *O. tauri*, including the effects of viruses, naturally biotinylated proteins and nutrients. Viruses which infect algae (Phycodnaviruses) play important roles in release of organic matter, nutrient cycling and horizontal gene transfer. Virus resistance has been reported in *O. tauri*. Here, a method for isolating resistant *O. tauri* cells from an infected population was developed by sorting cells which had low levels of DNA, with the hypothesis that cells which are infected by viruses will have more DNA. PCR results indicated that different strains may show resistance to viruses and that the virus DNA may have integrated into the host genome. Acetyl-CoA carboxylase is a biotinylated protein which plays a key role in fatty acid biosynthesis. Biotinylated proteins were enriched for in *O. tauri* in order to generate a list of these proteins which can later be used to understand more about lipid metabolism. Finally, the effect of different growth media on protein expression in *O. tauri* was examined. Results indicated that there were few significant differences in proteins between *O. tauri* grown in media with different nutrient compositions.
The Type VII/ESX-1 protein secretion system is found in many Gram positive bacteria and was first described in *Mycobacterium tuberculosis* where it was shown to secrete ESAT6 and CFP10. Both of these proteins are important T-cell targets and essential for the virulence of *M. tuberculosis*. A protein secretion system related to the ESX-1 protein secretion system in *M. tuberculosis* was later found and described in the human and animal pathogen *Staphylococcus aureus*. In the *S. aureus* Newman strain background the membrane-bound proteins EssA, EssB and EssC are essential for secretion of the substrate proteins EsxA and EsxB. The main focus of this study is to define the interactions between the membrane proteins of the ESX-1 machinery in *S. aureus*. To facilitate purification studies, it was first necessary to introduce an affinity tag onto each of the target proteins. It was shown that the presence of the engineered his-tags did not interfere with the secretion function of the ESX-machinery. Isolated *S. aureus* membranes were treated with a bidentate crosslinker followed by western blotting to identify possible interaction partners. High molecular weight crosslinked products could be detected for EsaA and EssC, but not for EssB. For further investigation, Blue Native (BN) PAGE gels were used to give better resolution of the high molecular bands and to identify native complexes. BN-gels do not contain SDS in the gel or in the running buffer. Therefore membranes were solubilised with a range of detergents to identify which would effectively extract the ESX membrane proteins.
Christopher Johnston  
The Mechanism and Functional Significance of Spliced Leader  
Trans-Splicing in Nematodes

Dr Jonathan Pettitt, University of Aberdeen  
Professor Bernadette Connolly

Spliced leader trans-splicing is a mechanism used by nematodes and several other invertebrate eukaryotic phyla to add a short RNA sequence called the spliced leader (SL) to the 5’ end of specific pre mRNA transcripts. Though SL trans-splicing is similar to cis-splicing of introns and some functions of it are known – such as its use in resolving polycistronic mRNA or operons – it is not well understood. If SL trans-splicing is used by a majority of nematodes, it would be a potential drug target for parasitic species. The free-living nematode Caenorhabditis elegans SL trans-splices 70% of its mRNA (>17% in operons) but it is not currently clear if other nematodes use it to this extent. We investigated the parasitic nematode Trichinella spiralis as it is phylogenetically diverse from C. elegans and thought to only SL trans-splice 1% of its mRNA. Using bioinformatic methods we identified 12 putative operons that are conserved in T. spiralis, C. elegans and Brugia malayi and therefore were likely present in the common nematode ancestor.
Bovine tuberculosis and Johne's disease are diseases of livestock caused by *Mycobacterium bovis* (*M. bovis*) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP), respectively. These two pathogens are genetically similar and cause similar pathology; both species are intra-cellular bacteria which infect at mucosal surfaces and are capable of surviving within host macrophages. Despite the bactericidal activity of macrophages, mycobacteria have evolved to survive within the macrophage itself where they are capable of driving granuloma formation and promoting mycobacterial survival. Activated macrophages can influence the adaptive immune response to mycobacterial infection through presentation of antigen, secretion of cytokines and expression of co-stimulatory molecules. As cell mediated immunity is required for protection, it is likely that the early response of the macrophage is central to infection outcome. This project aims to dissect interactions between the host and pathogen by assessing common and unique features between macrophage infection with *M. bovis* and macrophage infection with MAP. Bovine monocyte derived macrophages (MDM) have been isolated from peripheral blood mononuclear cells (PBMCs) and phenotyped using flow cytometry. MAP cultures have been grown and used to infect MDM; the infection has been quantified using confocal microscopy image analysis and colony counting. The phenotype of MDM was analysed over a time-course of infection (2, 6 and 24 hours), demonstrating that the infection altered the expression of cell surface molecules involved in the immune response. QPCR will be used to assess the expression of genes encoding key components of the immune response to mycobacterial infection over an infection time-course.
Due to a cycle of underreporting and lack of awareness, leptospirosis is now recognised as one of the world's most neglected diseases. The disease presents with a wide range of clinical signs in humans making clinical diagnosis difficult. In livestock the primary presentation is of reproductive losses making it a significant economic threat to the agricultural sector. Although no acute clinical case of leptospirosis has been diagnosed in Madagascar, other islands of the western Indian Ocean have some of the highest disease incidences in the world. Previous studies in Madagascar have identified potentially pathogenic *Leptospira* in introduced rodents, endemic small mammals and bats. Our aim is to understand the genetic diversity, and distribution, of *Leptospira* genotypes in Madagascar at varying spatial scales in a variety of hosts. This will allow us to elucidate the epidemiology of this multi-host pathogen in a region of high biodiversity. Previous studies have suggested host specificity in *Leptospira* genotypes maintained by endemic small mammal species and a lack of genetic diversity in *Leptospira* maintained by rats. However, our results indicate that, where endemic and introduced host species co-exist, cross infection does occur and that, consequently, *Leptospira* carried by rats demonstrate a wide genetic diversity. Potential implications of infections crossing the species barrier include the transmission of new pathogenic *Leptospira* strains to humans and livestock. Further work, using multi-locus genotyping schemes, will help to further refine the population genetic structure of *Leptospira* in Madagascar enabling the identification of possible transmission pathways.
Fraser Murphy1,2  
Characterisation of a host MAP3-Kinase targeted by an effector protein from the late blight pathogen, *Phytophthora infestans*

Miles Armstrong1, Hazel Mclellan1, Eleanor Gilroy2 and Paul Birch1,2.

*Phytophthora infestans* is the causal agent of potato late blight, a devastating disease that caused the Great Irish Famine during the 1840s. Today it cost six billion pounds to control and still poses a considerable threat to food security. Although plants are constantly under threat from microbes, the majority are unable to establish infection as they trigger host defence responses such as pathogen associated molecular pattern (PAMP) triggered immunity (PTI). *P. infestans* is able to manipulate many host signalling pathways through the secretion of effector molecules to evade plant defences. However, little is known about how these effector molecules manipulate host proteins to suppress defences from the plant.

We have recently identified a MAP3K, Vascular Highway 1 (VH1)/ Brassinosteroid Like 2 (BRL2)-interacting kinase (VIK), as the target of the *P. infestans* effector PITG_17316, via screening in a yeast-2-hybrid (Y-2-H) library of potato cDNAs. I will describe further Y-2-H, as well as Co-Immunoprecipitation, experiments that have been carried out to examine the specificity of the interaction between PITG_17316 with this particular MAP3K. Finally, transient expression of both PITG_17316 and VIK has provided evidence that defence pathways could be manipulated by this interaction.
Plant viruses are ubiquitous in natural growing environments and represent a constant and significant limitation to plant growth. This viral burden is of great economic importance due to the losses imposed by viral infections in crop plants. It has long been recognised that variations in environmental conditions can dramatically alter plant-virus interactions and may even dictate the outcome of infection. The light and temperature conditions plants are grown under have repeatedly emerged as important factors dictating both viral load and symptom development for a number of different phyto-viral systems. Potyviruses represent one of the two most specious groups of plant viruses and collectively present a large threat to agriculture. It has been well documented that Potyvirus infections become more severe with increasing temperature, though characterisation of this phenomenon remains scarce. Similarly, details of how Plant-Potyvirus interactions are affected by light conditions are ill defined. This poster will present initial results reflecting the influence these environmental factors have on the dynamics of Turnip Mosaic Virus (TuMV, a Potyvirus) in susceptible host plants.
The cost of reproduction is arguably the most prominent life history trade-off, with its principal prediction being that current reproduction is negatively correlated with survival and/or future reproduction. The environment, through its effects on resource availability, is likely to influence the existence or magnitude of life history trade-offs, however such evidence is limited, particularly for wild mammals. Using the long term data for the St. Kilda Soay sheep population, home range level vegetation information has been combined with life history data to show that ewes with greater access to the highly productive Holcus-Agrostis grassland experience reduced reproductive costs and bear lambs with improved survival probabilities. This suggests that the reproduction of female Soay sheep is condition dependent, with Holcus-Agrostis grassland enabling ewes to better cope with energetic losses resulting from reproduction. The association between lamb survival and ewe access to Holcus-Agrostis grassland may hint at differences in maternal provisioning with work currently underway to examine this.
Selection for growth has resulted in increased body size in meat type chickens but is associated with welfare and fertility problems. Understanding the genetics and physiology underlying selection for growth traits is critical for the future sustainability of the global poultry industry. Differential expression of the cholecystokinin A receptor (CCKAR) gene is associated with haplotype at the most significant quantitative trait locus for growth in chickens. CCKAR is the primary cholecystokinin (CCK) receptor involved in satiety, with activation affecting downstream regulation of feed intake and digestive activity. This project aims to uncover the genetic basis for differential CCKAR expression and further characterise avian CCK signalling. Results from genotyping the CCKAR locus in a segregating line have narrowed the region of potentially-causative variations to a focussed genomic segment spanning the gene. Sequencing across the CCKAR locus in high- and low-growth haplotypes has identified several hundred novel variations to be assessed for causation. Results from a 5’ RACE analysis of CCKAR mRNA disagree with the currently-accepted transcriptional start site position and work is underway to determine whether there is any difference between haplotypes. Elucidating the pattern and cause of differential transcription and expression of CCKAR gene will allow better understanding of genetic and physiological factors affecting the control of feed intake and growth, thus informing methods to control growth in fast-growing chickens.
The pineal gland is situated at the midline of the brain and produces melatonin, the hormone responsible for signalling darkness to the body and entraining daily physiological rhythms. Melatonin is a broadly acting hormone with receptors in peripheral organs and various central nervous system regions. Changes in melatonin production and signalling are associated with a number of diseases including type 2 diabetes and breast cancer, it is therefore important to understand how melatonin synthesis is regulated. The nightly production of melatonin is induced by upregulation of arylalkylamine N-acetyltransferase (AANAT) gene expression and activity, the rate-limiting enzyme for melatonin synthesis. Studies have shown there are high levels of retinol (vitamin A) in the mammalian pineal gland and that vitamin A deficiency causes a reduction in AANAT and melatonin levels. The effects of retinol are mediated by the active metabolite retinoic acid, a potent regulator of gene transcription. This project aims to confirm whether retinoic acid signalling components are present and function in the pineal gland and determine whether retinoic acid controls melatonin production. Organotypic culture of ex vivo rat pineal glands is being used to study this. We have found that retinoic acid receptors and key synthetic enzymes are present in the rodent and human pineal gland, some of which exhibit diurnal (day/night) changes in expression suggesting rhythmic production of retinoic acid in this gland. We have also shown that retinoic acid can upregulate Aanat expression, suggesting that retinoic acid has a role in the regulation of melatonin synthesis.
Many endogenous and exogenous compounds threaten the stability of our genomes by generating a broad spectrum of DNA lesions. To counteract such threats, organisms have evolved conserved DNA repair mechanisms that restore the original genetic information. However, mutations can still occur in the event of mutagen overexposure or error-prone DNA repair, giving rise to mutational processes. Mutational processes leave distinct imprints of mutations in the genome, whose nature depends on the mutagen, the local DNA chemistry and the genetic background of the organism. Actually, the overall mutational profile observed in a cancer genome results from the sum of all mutational signatures generated by DNA damage processes throughout cancer development. Despite whole genome sequencing (WGS) approaches having allowed researchers to decipher a large number of mutational signatures from cancer samples, the etiology of many patterns is still unknown. In this study, I employ the nematode *Caenorhabditis elegans* as an intoxication model to reveal mutational signatures caused by known and suspected carcinogens. Wild-type and DNA repair-deficient worms are exposed to mutagens and their genomic DNA is sequenced by WGS. Bioinformatic analysis will extrapolate mutational signatures. As DNA repair pathways are well conserved between *C. elegans* and humans, this study will allow us to link specific patterns of mutation found in human cancers with those arising in worms, hence, providing information on the environmental and genetic factors that contribute to the development of such tumours.
Many pathogens cause plant diseases that can decimate yields of important food crops. Increasing pathogen resistance to current treatments has resulted in an urgent requirement for effective alternatives. Biological control is one such promising alternative whereby microorganisms can be used to prevent plant diseases. The formation of biofilms on plant roots by microorganisms is often vital for biocontrol. Biofilms are complex multicellular communities of bacteria interacting with each other, and a surface, and encased in a self-produced extracellular matrix. The matrix is composed of exopolysaccharides, proteins and in some cases nucleic acids, and provides protection to the cells from various stresses. One such microorganism that relies on biofilm formation for biocontrol, is the Gram-positive soil-dwelling bacterium *Bacillus subtilis*. Biofilm formation in *B. subtilis* is tightly regulated by signalling networks that ultimately result in the production of two essential matrix components; exopolysaccharides and an amyloid protein (TasA), in addition to a bacterial hydrophobin (BslA). Previous work has shown that TasA and EPS are required for biofilm formation and biocontrol by *B. subtilis* on many different plant species. However, no previous work has looked at the possible role of BslA in plant-related biofilm formation. In order to investigate this we have developed an assay to quantify levels of root colonisation by *B. subtilis* on the tomato plant and have confirmed the importance of EPS and, in particular, TasA on root colonisation. Additionally, we have found that BslA does not appear to be involved in plant root colonisation at early time points. However this does not preclude a role for BslA in persistence of colonisation or biocontrol.
Selectins are cell adhesion proteins which support the response of mammalian immune system to inflammation and injury. These proteins regulate homing of leukocytes to the peripheral lymph nodes, where they encounter antigens and become activated, and from there to the inflamed tissues where they can actively protect organism from damage. They also mediate leukocyte binding to platelets during the thrombi formation and are believed to play important role in other processes which involve cell adhesion, such as the tumor cell metastasis.

To achieve their physiological function, selectins bind to glycoproteins which carry specific modifications, such as sialylation, fucosylation and sulfation. Recent findings from our group show that L- and P-selectins display high affinity binding to a fucosylated chondroitin sulfate (fCS) polysaccharide extracted from the sea cucumber species *Holothuria forskali*. The two selectin domains that play major roles in the ligand binding processes are the C-type lectin domain and the EGF-like domain. Efficient expression of these two domains in the yeast *Pichia pastoris* would allow for production of isotopically labeled protein constructs which could be used in structural Nuclear Magnetic Resonance (NMR) studies on the interaction of selectins with fCS. Preliminary work indicates that the one- and two-domain L- and P-selectin constructs can be produced with the use of *P. pastoris* expression system. Current experiments focus on the development of the optimised protocol for purification of the selectin constructs from *P. pastoris*.
The RNA interference (RNAi) pathway employs double stranded RNA molecules that are processed by the ribonuclease protein Dicer (Dcr1) into short-interfering RNAs (siRNAs). In fission yeast, siRNAs are loaded onto the protein Argonaute and facilitate the recruitment of protein complexes that can nucleate heterochromatin via modification of the histone tails. I aim to investigate differences in the role of the RNAi pathway between the extensively studied S. pombe and the relatively uncharacterised S. japonicus. These two fission yeast species are highly diverse, allowing us to ask to what extent the pathways characterised in S. pombe are conserved. Of particular interest is the difference in transposon complement between the two species; S. japonicus has 10 families of retrotransposons, whilst S. pombe has only two. RNA-Seq data has shown that the S. japonicus transposons produce high levels of siRNAs, but whether these act via the RNAi pathway to nucleate heterochromatin and regulate transposition is unknown. RNAi plays a minor role in transposon silencing in S. pombe; instead the Cbp1 complex is utilised. The appearance of these proteins coincides with a dramatic decline in the number of transposons across the fission yeast species. This complex is lacking in S. japonicus; this questions what plays this role in S. japonicus and whether this is analogous to the RNAi-based method that S. pombe employs to silence centromeric repeats. As S. japonicus transposons are found in similar genomic positions to the S. pombe centromeric repeats, it may be that they play a functionally equivalent role.
Under natural selection, genetic diversity is usually reduced at functional loci, and yet the major histocompatibility complex (MHC) remains highly polymorphic, likely due to balancing selection. The MHC encodes molecules which detect parasites and trigger an immune response; thus parasites are likely to be important drivers of selection at the MHC. The exact mechanism by which parasite-mediated selection (PMS) maintains high polymorphism, however, remains highly debated. The St. Kilda Soay sheep (*Ovis aries*) are an excellent study system for investigating this in a non-model organism as they are unmanaged and therefore subject to natural selection, and individual level data are available on fitness, parasitological and immunological measures across temporal and spatial scales, which will be useful in discriminating between modes of PMS. Using genotyping-by-sequencing techniques developed for domestic sheep, we were able to generate high-quality genotypes for the MHC class II. DRB1, the most polymorphic locus, was genotyped in 28-30 individuals from each of four cohorts (1993, 1998, 2003, and 2008) and revealed six alleles. High linkage disequilibrium was expected between the class II loci, thus DRB1 homozygous individuals were genotyped at DQA and DQB loci, revealing seven haplotypes; a single DRB1 allele was shared across two DQA and DQB haplotypes. Knowledge of these haplotypes should enable rapid imputation of genotypes across the Soay sheep pedigree. Next generation sequencing will be used to genotype class I alleles, and then associations between MHC diplotypes and fitness measures, parasite burdens and immunological measures will be investigated.
The microbiota is composed of a diverse range of microorganisms which occupy different sites in the body including the gut, mouth, skin and genitals. The lung was previously thought to be sterile and thereby harbour no microbiota of its own. However, this idea has recently been questioned through the use of non-culture based techniques such as 16S amplicon sequencing. Several non-infectious diseases have been related to changes in the lung microbiota such as cystic fibrosis, chronic obstructive pulmonary disorder and lung transplant rejection.

This study seeks to characterise the bacterial lung microbiota in sheep: its composition, how it is established and how it is related to disease. Brushings were taken from the lungs of healthy sheep over three time points (baseline, one month and three months) to discover whether there is a stable, individually specific lung microbiota. Lamb lung fluid samples were also collected to determine whether bacterial DNA in the lungs originates from live or dead bacteria.

Samples taken from lung fluid and lung epithelium brushings contained significantly higher concentrations of bacterial DNA than negative controls. Bacterial communities sampled from different lung sites and the first two time points were found to significantly cluster by the sheep from which they were taken. Samples were also found to significantly cluster by time point. In order to attempt to separate the effect of time point and sampled individual the three month time point will also be analysed.

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In a 2006, the ability of 4 transcription factors Sox2, Klf4, Oct4 and c-Myc to ‘reprogram’ differentiated somatic cells back to a pluripotent state was demonstrated. This technology has huge potential in the field of regenerative medicine, but reprogramming also offers a model by which the cascade of cellular events that result in such a drastic alteration of cell identity can be investigated. Despite this however, the mechanism of reprogramming still remains poorly understood. One powerful method for elucidating the genetic components involved in a biological process such as reprogramming is screening for desired phenotypes using genome-wide mutant libraries. However, knockout screens can be difficult to perform in diploid mammalian genomes while other screening technologies such as RNAi have disadvantages such as insufficient knockdown of transcripts and off target effects.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs)/Cas technology has been recently adapted to cultured mammalian cells from a prokaryote form of adaptive immune system, demonstrating a capacity to introduce site specific knockout mutations with high efficiency. Furthermore, the scalability of CRISPR/Cas technology to a genome-wide level has now been demonstrated.

We therefore aim to utilise recent advances in knockout generation technology to perform genome-wide knockout screening to dissect the molecular mechanisms underlying reprogramming.
The floral promoter FPA has been implicated in the processing of a number of mRNAs. Recently we have shown that FPA co-localises in vivo with a large number of proteins, including two members of the RNA N-6-methyltransferase complex; MTA and MTB. We have identified several proteins which show interaction with FPA in Yeast-2-Hybrid (Y2H), including MTB. The same in vivo technique has been applied to MTA in overexpression plants, and has allowed the identification of a putative five-protein methyltransferase complex, which is being tested in Y2H.