QMB Abstracts

Speaker Abstracts Q1-Q23

Q1: Imaging and modelling malaria parasites

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Plasmodium falciparum is the most virulent of malaria parasites, causing ~440,000 deaths per year. Efforts to control malaria need to target both intraerythrocytic asexual multiplication, which causes disease; and sexual development, which is responsible for transmission.

New microscopy techniques are providing amazing views of the cellular landscape. We have used 3D Structured Illumination Microscopy, direct Stochastic Optical Reconstruction Microscopy, Block-Face Scanning Electron Microscopy and 3D-Electron Tomography to explore the sub-cellular topography of *P. falciparum*.

We have imaged modifications made to the host red blood cells (RBC) membrane, including PfEMP1 trafficking and knob assembly, which facilitate cytoadherence in asexual stages. We have examined the assembly of the parasite cytoskeleton and membrane complex, and the reorganisation of the host RBC membrane, which underpin the remarkable transformation to the gametocyte stage, in preparation for transfer to a mosquito and sexual reproduction.

We have developed a computationally-efficient coarse-grained model of the RBC membrane and show that the increase in the rigidity of the host RBC membrane in trophozoites and mid-stage gametocyte, is mediated by restructuring and constraining the spectrin meshwork, as well as by enhanced vertical interactions that lead to strain-hardening and/or entropic effects (Dearnley *et al.*, 2016, Zhang *et al.*, 2015). We examined the increase in deformability in stage V gametocytes that facilitates passage through the sinusoidal slits in the spleen, and helps avoid host surveillance mechanisms.

Q2: Host actin filament-stabilising protein, cortactin promotes influenza virus infection, but undergoes caspase-mediated degradation in epithelial cells

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Influenza virus has been a highly successful human respiratory pathogen. A comprehensive understanding of influenza virus-host interactions is needed to develop effective and long-lasting influenza vaccines and antiviral drugs. Influenza virus is known to exploit host factors to its advantage. Here, we report that host cortactin, an actin filament-stabilising protein and a substrate of recently-described anti-influenza host factor, histone deacetylase 6 promotes influenza virus infection in epithelial cells. However, cortactin also undergoes the degradation during late in infection in a strain-independent manner. We found that RNA interference-mediated knockdown of cortactin expression decreased influenza virus infection by up to 59%, whereas the overexpression of cortactin increased it by up to 60%. Interestingly, the level of cortactin polypeptide, but not mRNA was found to be downregulated by up to 84% after 24 h of influenza virus infection. A lysosomal inhibitor, NH4Cl reversed the effect of influenza virus infection and rescued the level of cortactin polypeptide in infected cells. In contrast, a proteasomal inhibitor, MG132 exacerbated cortactin downregulation in infected cells and even induced it in uninfected cells. This indicated an involvement of caspases in cortactin downregulation as MG132, like influenza virus is known to induce apoptosis. Indeed, a caspase 3 inhibitor rescued cortactin polypeptide level similar to the level of NH4Cl treatment. However, we did not detect cleaved cortactin fragments in infected cell lysates by western blotting using antibodies recognising both N-terminal/Central and C-terminal cortactin regions, suggesting the presence of multiple caspase cleavage sites. Indeed, CaspDB, a recently-described database predicted up to 35 caspase cleavage motifs spread throughout cortactin polypeptide. The data obtained indicate that a lysosomal-associated apoptotic pathway mediates the degradation of host cortactin, which potentially has a dual but contrasting role during early and late stages of influenza virus infection.

Q3: Long noncoding RNA *ZFAS1* in ribosome regulation: a riddle wrapped in a mystery inside an enigma

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Ribosomes, central to protein synthesis in all cells, are complex multicomponent assemblies involving hundreds of molecular components in their function and biogenesis [1]. Despite being discovered in the mid-1950s and the subject of intense interest, much surrounding the regulation of the ribosome and its biogenesis remains unknown.

In this study, we propose that the lncRNA *ZFAS1* has a novel role in ribosome biogenesis. *ZFAS1* was originally discovered by microarray analysis in mouse mammary gland development [2]. We have shown in both mouse and human that the *ZFAS1* transcript is present in both cytoplasm and nucleus in an isoform-independent manner. Despite lacking coding potential, we have found that *ZFAS1* is associated with polysomes in the cytoplasm. When ribosomes are dissociated, *ZFAS1* remains associated with the small 40S subunit [3].

Analysis of mouse microarray data from our previous study of mouse mammary glands [2] identified five ribosomal proteins (rps3, rps21, rps24, rpl22, rpl28) with the same expression pattern as *Zfas1*. These ribosomal proteins have roles in ribosome production, assembly, and maturation. TCGA gene expression data confirmed similar expression for *ZFAS1* and nominated ribosomal proteins in human normal and breast cancer samples.

Further studies in models of ribosome biogenesis found that expression of *ZFAS1* is induced after exercise in human muscle cells [4]. Its upregulation is concordant with that of 45S rRNA, suggesting it may function in ribosome biogenesis. Mouse IGF overexpressing and myostatin knockdown$^{-/-}$ models exhibit larger muscle mass compared to WT controls. Increased muscle mass requires higher rates of protein synthesis and elevated levels of ribosomes. *ZFAS1* expression is correlated with this increased muscle mass as well as with total RNA concentrations, providing further evidence that *ZFAS1* may be involved in ribosome regulation.

Currently we are investigating the role of *ZFAS1* in pull down systems and using CRISPR/Cas9 to knock down *ZFAS1* to explore its role in this complex process.

1. Thomson, E., et al., (2013) J Cell Sci. 126, 4815-21
2. Askarian-Amiri, ME., et al., (2011) RNA 17: 878-91.
3. Hansji, H., et al., (2016) RNA Biology (manuscript submitted).
4. Figueiredo, VC., et al., (2016). Physiol Rep. 4(2). pii: e12670.

Q4: Single-molecule studies of DNA replication: the plasticity of the replisome

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Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual enzymes and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these enzymes function in concert with other enzymes in multi-protein complexes, so an important new direction is the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies.

We are applying a single-molecule approach to study DNA replication, a process that is supported by a large, multi-protein complex containing a number of different activities and held together by protein-protein and protein-DNA interactions of many different strengths. While the classical textbook picture of the replisome is one in which the protein components, in particular DNA polymerases, are efficiently retained and re-used for a large number of cycles of Okazaki-fragment synthesis on the lagging strand, our single-molecule studies imply a very different picture. Using model DNA-replication systems derived from *E. coli* and T7 bacteriophage, I will present fluorescence-based single-molecule imaging experiments demonstrating that new DNA polymerases are recruited by the replisome on a timescale of mere seconds. Further, simultaneous observation of both leading- and lagging-strand synthesis in a single replisome suggests that the replication machinery employs a number of different molecular mechanisms to achieve coordination between the DNA polymerases on each strand.

Q5: Emergence of RNA editing in a long term evolution experiment

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RNA editing, the correction of genomic errors by altering the sequence of messenger RNA, has evolved multiple times independently, but it remains unclear exactly how such a process has evolved. A model for the emergence of RNA editing proposed that RNA editing activity pre-exists but there is no substrate for editing activity to act upon. Subsequently, mutation creates editable nucleotide sites, which may be fixed by genetic drift making RNA editing indispensable for expression of functional genes[1]. We sought to test this model by asking whether a class of slippage-type editing can evolve under experimental conditions designed to maximize the effect of genetic drift.

We previously showed that, in the bacterial endosymbiont *Buchnera*, RNA polymerase slips at long poly(A/T) tracts, leading to stochastic incorporation or removal of As or Us in the nascent messenger RNA. This results in a heterogeneous population of mRNAs. Slippage-type editing was shown to correct errors in genes which had acquired natural frameshift mutations, but this may in turn reduce expression efficiency of genes with intact reading frames[2].

In a long-term evolution experiment using *Escherichia coli*, we subjected 10 lines to daily single-cell bottlenecks. Following 50 days of bottlenecking, one line showed an observable reduction in growth rate. Genome sequencing revealed the emergence of 38 frameshift mutations that require slippage-type editing for gene expression. We present data showing that slippage-type editing rescues frameshift mutations but that protein production is reduced. Our results support the hypothesis that, under conditions favouring genetic drift, editing readily emerges. To our knowledge, this is the first experimental demonstration of the evolutionary drivers for the emergence of RNA editing.

1. Covello, P.S. & Gray, M. W. (1993) *On the evolution of RNA editing*. *Trends Genet.*, 9(8), 265-268..
2. Tamas, I. *et al.* (2008) *Endosymbiont gene functions impaired and rescued by polymerase infidelity at poly(A) tracts*. *Proc. Natl. Acad. Sci.*, 105**,** 14934-9

Q6: The secret life of kinases: insights into non-catalytic functions from pseudokinases

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While protein kinases are best understood for their phosphoryl transfer enzymatic activities, recent mechanistic studies have led to recognition of non-catalytic functions in cell signaling. Studies of pseudokinases - the catalytically-impaired or dead cousins of conventional protein kinases - have been instrumental in shining light on the vast, varied and ever-expanding repertoire of mechanisms by which kinase-like domains can mediate protein interactions to modulate cellular signaling.

Although best characterized as allosteric regulators of conventional, active protein kinases, our work and that of others suggests more broadly that pseudokinase domains are important protein interaction modules that can function as molecular switches, scaffolds and adaptors in cell signaling pathways. Here, I will describe how our work has attributed these functions to the pseudokinase, Mixed lineage kinase domain-like (MLKL). Overall, these studies suggest how conventional protein kinases could moonlight as protein interaction modules, scaffolds or molecular switches to complement their better-understood catalytic roles.

Q7: Single-particle cryo-electron microscopy - coming of age?

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It is about twenty years since the first three-dimensional (3D) reconstructions at subnanometer (< 10 A) resolution of an icosahedral virus assembly were obtained by cryogenic electron microscopy (cryo-EM) and single-particle analysis. Since then, hundreds of structures have been determined to resolutions ranging from 30 A to near-atomic (< 4 A). The recent development of direct electron detectors, and the attendant improvement in analysis software have, almost overnight, pushed the technology a major step forward. Near-atomic resolution reconstructions can now be obtained, not only for large MDa macromolecular complexes or highly symmetrical assemblies, but also for proteins of only a few hundred kDa. I will discuss the developments that led to this breakthrough in high-resolution structure determination by cryo-EM, but also point to many challenges that lie ahead.

Q8: Mutational robustness of RNA and protein: transitions from an RNA world

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Biological systems need to be replicated with sufficient fidelity to preserve their function. The level of fidelity required likely depends on different factors, including what types of biomolecules (RNA, protein, etc) are used in that system. The first biological replicators likely had low fidelity: as replication fidelity increases, different systems can evolve. The ability of a biomolecule to maintain function in the face of mutation is known as mutational robustness. How RNA and proteins differ in mutational robustness might effect the transition from one to another during the early evolution of life.

We are interested in how RNA and protein differ in mutational robustness at the information (DNA) level. Few direct comparisons have been made between the robustness of RNA and proteins, and these have been primarily based on highly simplified computational models. We are comparing the robustness of RNA and protein experimentally using mutant libraries of the fluorescent RNA broccoli and fluorescent protein mCherry. Sequence data from individual mutants can be correlated to their respective level of function, thereby linking the genotypes to the fluorescent phenotype.

Combined with our computational analyses, this preliminary work indicates that while RNA and protein differ in their response to mutation, one may not be more robust under all conditions than the other. This opens up a rich field of investigation into RNA and protein robustness, which can inform our understanding of the early evolution of life. Assuming an early RNA world, if protein is much less robust than RNA and more sensitive to mutation, a high fidelity polymerase would need to evolve before proteins could take over RNA functions. If proteins have comparable robustness, this transition could occur earlier.

Q9: Circumventing the stability-function trade-off in protein engineering

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The favorable biophysical attributes of non-antibody scaffolds make them attractive alternatives to monoclonal antibodies. However, due to the well-known stability-function trade-off, these gains tend to be marginal after functional selection. A notable example is the fibronectin type III (FN3) domain, FNfn10, which has been previously evolved to bind lysozyme with 1 pM affinity (FNfn10-α-lys), but suffers from poor thermodynamic and kinetic stability. To explore this stability-function compromise further, we grafted the lysozyme-binding loops from FNfn10-α-lys onto our previously engineered, ultra-stable FN3 scaffold, *FN3con*. The resulting variant (FN3con-α-lys) bound lysozyme with a markedly reduced affinity, but retained high levels of thermal stability. The crystal structure of FNfn10-α-lys in complex with lysozyme revealed unanticipated interactions at the protein-protein interface involving framework residues of FNfn10-α-lys, thus explaining the failure to transfer binding via loop grafting. Utilizing this structural information, we redesigned FN3con-α-lys and restored picomolar binding affinity to lysozyme, whilst maintaining thermodynamic stability (with a thermal melting temperature two-fold higher than that of FNfn10-α-lys). FN3con therefore provides an exceptional window of stability to tolerate deleterious mutations, resulting in a substantial advantage for functional design. This study emphasises the utility of consensus design for the generation of highly stable scaffolds for downstream protein engineering studies.

Q10: ThermoFisher Scientific Award: Molecules in motion: computational molecular biology

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In order to understand biological function, it is essential to understand how biological molecules move and interact. Molecular dynamics simulations have the capacity to provide this information, as well as elucidate the underlying energetic driving forces, at an atomic level of detail. I will briefly explain how molecular dynamics simulations work, including their strengths and limitations. I will then illustrate how simulations can provide insight into a range of biological phenomena that could not otherwise be obtained, from understanding the molecular basis of Parkinson's disease to improving detection of evolutionary arms races.

Q11: Riding the structural biology wave: towards an atomic view of biology

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Lawrence Bragg described the excitement of the early days of X-ray crystallography, 100 years ago, as being "like discovering a new goldfield where nuggets could be picked up on the ground, with thrilling new results every week". He was referring to the crystal structures of simple salts and minerals. Biologists had to wait 50 years for a similar revolution, but the past 50 years have seen enormous advances in our ability to visualize biological systems in atomic detail. In this talk I discuss the origins of structural biology in New Zealand, identify important developments along the way, and look to the future of this key part of biological research, debunking a few myths along the way.

It may seem strange from today's vantage point, but there were strong views that protein crystallography should not be pursued in this part of the world. I will describe the importance of mentors in overcoming this view, and the approaches that had to be taken; very much "do-it-yourself". I will then go on to highlight a few of the "gold nuggets" that our research has uncovered. In one example, crystal structures of domains from the extended protein assemblies (pili and adhesins) through which bacteria attach themselves to human cells have revealed the presence of autocatalytically-formed covalent cross-links. These linkages not only explain how these long, thin proteins are stabilized, but also offer new tools for applications in biotechnology. In another, our research into an important biosynthetic enzyme and drug target from *Mycobacterium tuberculosis* has enabled us to trap each of the key intermediates of its catalytic cycle, defining its mechanism in atomic detail. Finally I will highlight the recent spectacular work of PhD student Jason Busby on an insecticidal toxin that points to the growing convergence of crystallography and cryo-electron microscopy.

Q12: Developing next-generation solar powered microalgae systems for the production of high value products, foods and renewable solar fuels

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The global economy is valued at ~$110 Tn and is expected to grow significantly as our population rises from 7.4 billion towards 9.6 billion people by 2050. This increase in population is forecast to require approximately 70% more food (UN), 50% more water (OECD), 50% more fuel (International Energy Agency) and 50-80% reductions in CO_2 emissions (Intergovernmental Panel on Climate Change) to maintain political, social, fuel and climate security. While, these challenges highlight important growth opportunities, they must be achieved sustainably for a secure future.

Microalgae are positioned at the nexus of these challenge as they tap into the huge energy resource of the sun (~2300× global energy demand), capture CO_2 and can expand photosynthetic capacity into the oceans or onto non-arable land using saline water. The captured solar energy and CO_2 are used to produce a broad range of biomolecules which collectively form biomass. Through bio-refinery processes these natural or engineered biomolecules can be separated into high value products (e.g. recombinant proteins >$1000 kg^{-1}), nutraceuticals (e.g. anti-oxidants, unsaturated fatty acids), foods/feeds and CO_2 neutral renewable fuel (e.g. crude oil, biodiesel, methane, ethanol and hydrogen, <$300 Ton^{-1}). From a climate change perspective, CO_2 neutral solar fuels are critically important as 80% of global energy demand is provided as fuels and only 20% as electricity. Despite the fact that we must achieve CO_2 emissions reduction of ~50% by 2030 to stay within global warming 'safe zone', to date we have no demonstrated sustainable large scale renewable fuel solutions. To fast track solar fuel production we are focused on developing economic opportunities down the cost curve by working from high value products towards fuels, to increase industry and government investment and international collaborations.

In this talk I will present our process pipeline for the development of next generation microalgae systems. In broad terms this pipeline includes molecular, structural and cell biology as well as process engineering. Specifically it incorporates high-throughput processes for the purification of native microalgae strains, efficient cryo-preservation protocols, robotic nutrient and light optimization screens, nuclear and chloroplast engineering strategies, recombinant fusion protein production, pilot scale systems design as well as techno-economic and life-cycle analyses to guide next-generation system development and scale up.

A particular focus of our work is on the structure guided optimization of light capture efficiency of the thylakoid solar interface (cryo-electron microscopy, single particle analysis and electron tomography, nuclear engineering and RNAi) and high-throughput chloroplast expression of recombinant proteins (e.g. for the production of vaccine antigens and peptide bioactive molecules). Through this structural work we are focused on developing atomic-resolution 3D atlases of the exquisite and dynamic photosynthetic machinery. These are designed to reveal geometric arrangements of the photosynthetic complexes within the thylakoid membranes towards atomic detail. Such atlases provide a valuable blueprint to guide the targeted engineering of high-efficiency microalgae cell-lines for next-generation

solar driven bio-technologies. They also provide valuable insights into 3 billion years of evolutionary refinement for the bio-inspired design of artificial solar technologies.

Q13: Fucoxanthin as a potential healthy and functional food ingredient: increasing production, yield and range of bioactives from microalgae and cyanobacteria

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Microalgae produce many valuable molecules that can be used as ingredients in healthy and functional foods, as nutraceuticals, cosmeceuticals and as biochemical reagents. These often have antioxidant, anti-inflammatory and other bioactive properties, but are usually present in low amounts. This talk will describe methods to increase the range and yield of bioproducts from algae and cyanobacteria.

The Cawthron Institute holds the New Zealand national microalgal collection, which includes over 600 algal and cyanobacterial strains from diverse locations across the Pacific, New Zealand and Antarctica. Cawthron also operates a shellfish hatchery that produces monocultures of microalgae in an enclosed continuous bag system as feed for shellfish larvae and an extensive open pond system for juveniles and broodstock.

Cawthron has developed a multi-vessel photobioreactor system to improve the yield of target molecules from microalgae and cyanobacteria. The system, used in conjunction with a rigorous experimental design matrix, allows multivariate optimisation of growth parameters for product formation and yield. Light, temperature and pH are monitored and controlled individually. When manipulated with various media changes, the optimal conditions for a given bioproduct can be determined rapidly.

We have used this system to isolate and optimise production of different bioactives (e.g. carotenoids such as fucoxanthin) from different algal strains. Some of these products have been evaluated in an *in vitro* model of inflammation and an *in vivo* model of allergy with positive results.

Q14: Microbial seafloor response to the Gulf of Mexico oil spill

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The Deepwater Horizon oil spill led to persistent petroleum pollution of deep-sea sediments entrained with alkanes and (poly)aromatic hydrocarbons. To better understand the largely enigmatic seafloor community response, we reconstructed 57 uncultivated bacterial genomes post spill, and recovered their spatial gene expression patterns. The genomes were observed across 13 seafloor locations, 0.3 to 59.5 km from the damaged wellhead, and were highly enriched towards point source, revealing a predominance of oil spill responsive bacteria even in locations not previously recognized as spill impacted. Reconstructed genomes also exhibited significant phylogenetic clustering indicative of trait selection. We identified 273 genes associated with hydrocarbon degradation from 28, mostly gammaproteobacterial genome bins. All were potential alkane degraders, and a small yet significant subset harbored elaborate gene inventories for degradation including multiple aromatic-ring-hydroxylating dioxygenases. Differential gene expression at proximal versus distal sites was driven by largely distinct groups of these widespread bacteria. Gene up-regulation near the wellhead indicated nitrate- and oxygen-dependent sulfur oxidation, sulfate reduction, and the removal of short-to-mid length n-alkanes and (poly)aromatic hydrocarbons were all fuelled by residual oil pollution. Results point to a common collection of oil-responsive bacteria, replete with hydrocarbon degradation strategies, that exhibit spatially differentiated metabolic responses.

Q15: Enhancing environmental biomonitoring using high-throughput sequencing

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Recent technological advances in molecular methodologies provide opportunities to develop innovative diagnostic tools that can streamline and reduce costs associated with biological monitoring. High-throughput sequencing (HTS) is a relatively new technique that can cost-effectively produce large volumes of DNA or RNA sequence data. This provides the potential for sensitive and rapid detection of all organisms present in any given environmental sample (water, soil, sediment, etc.). The technique can be applied to a huge range of organisms expanding the scope of monitoring programs into biota and/or habitat groups that are currently not being surveyed due to technical limitations or poor taxonomic knowledge. Two ongoing research projects will be showcased to illustrate the pros and cons of using HTS for monitoring aquatic ecosystems in New Zealand. The first project investigates the use of HTS for the detection and characterisation of marine invasive species. The second project explores the capacity of HTS for environmental monitoring of bio-indicator bacteria, eukaryotes and foraminiferal communities around fin-fish farms. HTS-based tools have tremendous potential for improving New Zealand's environmental management of a variety of freshwater and marine ecosystems.

Q16: Plankton to Pooh: What metabarcoding can tell us about whales and their diet

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Ecology has gradually shifted away from the idea that ecological systems are balanced and stable, towards an understanding of ecosystems as dynamic and governed by complex processes. In the highly dynamic marine environment it's challenging to quantify seemingly simple ecological relationships such as predator-prey interactions using traditional methods. The development of molecular tools such as metabarcoding, now allow us to determine trophic interactions and the influence of environmental changes on such interactions. We have used a multi-locus metabarcoding approach to understand the diet of the largest predator residing in the Hauraki Gulf, Auckland, the Bryde's whale, against a backdrop of the potential food that's available. We amplified and sequenced 18S, 16S and COI DNA barcodes of the zooplankton community from three ecological regions throughout the Gulf (n = 24) and collected, amplified and sequenced whale scat (n = 20) to determine whether whales opportunistically foraged or actively selected preferred prey throughout the year. A single MiSeq run produced over 14 million paired end reads across all three DNA barcodes resulting in 6,156 OTUs for 18S, 733 for 16S and 8,319 for COI. There was higher plankton biodiversity in the warm-water season and in the outer Gulf, with decreasing diversity towards the Firth of Thames. Taxa represented in high abundance in the whales' diet included copepods, krill, salps, ray-finned fishes and planktonic crustaceans. Seasonal and geographic patterns in zooplankton diversity and diet did not match, strongly suggesting that whales are preferentially selecting prey independent of availability. These tools will be used to inform how they identify their prey and how changes in prey availability may affect the long-term viability of New Zealand's largest resident mammal.

Q17: Illumina Emerging Researcher Award: Whole-transcriptome profiling of flexible sexual phenotypes in a model sex-changing fish, the bluehead wrasse

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Sex is increasingly seen as a continuous, rather than a dichotomous trait. Sex is phenotypically plastic in many marine fishes and results from environmentally-sensitive differential gene regulation. Bluehead wrasse (*Thalassoma bifasciatum*) are highly-social reef fish and well-studied models of sexual plasticity. These diandric, protogynous (female-first) hermaphrodites have three sexual morphs as adults whose development is plastic and socially cued. Bluehead wrasse mature as male (primary males) or female, but each have the capacity to become dominant (secondary) males later in life. Large, brightly coloured secondary males actively defend and court a harem of females, whereas primary males are female-mimics that employ a 'sneaker' mating strategy. Using whole-transcriptome RNA-sequencing (RNA-seq) we have explored the molecular basis of plastic sexual phenotypes in the bluehead wrasse brain and gonad. Differential expression analysis identified thousands of genes important in the maintenance of the primary male, secondary male, and female phenotypes. In the brain, secondary males had the most distinct expression patterns, whereas expression profiles of primary males reflect their female-like behaviour, not their male sex. We find that isotocin (homologue of mammalian oxytocin) is overexpressed in secondary males, supporting recent evidence for its regulatory role in teleost social interactions, especially those related to dominance and rank. Gonadal expression profiles were strongly sex-biased, although secondary males upregulated genes involved in androgenesis and in the maintenance of secondary sexual characteristics (i.e., colouration and territoriality). Further investigations into the molecular basis of sexual plasticity are now underway, including transitions between alternative phenotypes and comparisons of gene expression patterns in evolutionarily divergent systems.

Q18: Evolutionary Genomics of Adaptation to Environmental Change in Non-model Aquatic Organisms

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Understanding whether natural populations will be able to adapt to selective pressures associated with rapid environmental and climatic change is a research priority. Measuring the strength and characteristics of selection in natural populations remains a daunting task, particularly for non-model species. In this talk I present results (and several unresolved challenges) from three research programs that study adaptation in non-model marine and freshwater organisms by integrating population genomics with environmental modelling and common-garden experiments. Each program explores natural replicates of the adaptation process by comparing closely related lineages and populations in geographically separate environments or in shared environments. Our datasets include ddRAD, RNA-seq, candidate adaptive genes, habitat mapping and trait phenotyping. The main aim of the first program (23 populations of two species of abalones, n=732) was to assess the relative contributions of space and selection in large, well-connected marine populations. Substantial neutral gene flow was the norm in both species, but their adaptive datasets showed marked population structure associated with environmental heterogeneity; in particular, with thermal gradients. In the second program (50 populations of two perch species, n=638), we tested for associations between neutral and adaptive diversity and gradients of environmental disturbance. Both species showed neutral population structure linked to the riverine network. However, it appears that long-term environmental instability (measured by natural hydroclimatic disturbance) has promoted adaptive diversity and evolutionary resilience in these lineages. In the third program (55 riverine populations of three rainbowfish species, n=1305) we experimentally assessed adaptive potential to climate change and tested landscape predictions from the 'climatic variability hypothesis'. We showed heritability and heritable plasticity for the expression of candidate genes in future climates. At a landscape level, populations from more variable habitats showed higher adaptive resilience to climate change. Strategies for cataloguing adaptive resilience to environmental change in ecologically important organisms are discussed.

Q19: Rapid adjustment of ejaculate quality in response to sperm competition risk

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Chinook salmon (*Oncorhynchus tshawytscha*) form social hierarchies, in which dominant males guard females, with subdominants displaced. Subdominant males commonly invade spawning pairs, resulting in sperm competition wherein sperm from many males compete to fertilise the same ova. Recent work shows that males with faster sperm outcompete rivals and sire a higher proportion of eggs [1,2], while models[3] predict subdominant males compensate for poor mating position by producing higher quality ejaculates (i.e., more and faster sperm) compared to dominant males. These same models predict males will maximise fitness by adjusting ejaculate quality in response to change in social position, with some empirical proof now collected[4]. How such rapid adjustments might be made is unknown, but alterations to seminal fluid protein (SFP) composition appears a likely candidate mechanism for increasing sperm velocity[5].

We utilised a repeated measures design, in which we paired males to determine social status, then re-paired males of the same status, forcing one male in each pair to change. At each experimental stage, we measured sperm concentration and sperm velocity, recombined ejaculates using sperm and seminal fluid from different males to determine the influence of seminal fluid on sperm velocity, and compared SFPs using Mass Spectrometry. Our results demonstrate: (1) subdominants produce higher quality ejaculates than dominant males, (2) males are able to make rapid adjustments to ejaculate quality in response to changes in social status and (3) seminal fluid has a direct effect upon sperm velocity. We present results comparing SFPs among males with the aim of producing a candidate list of SFPs that may modify sperm velocity. Our combined results indicate chinook salmon invest in seminal fluid to increase competitiveness of sperm based on sperm competition risk. This research highlights the role of seminal fluid in the rapid adjustment of sperm performance.

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2. Rosengrave, P., Montgomerie, R. & Gemmell, N. (2016) *Cryptic female choice enhances fertilization success and embryo survival in chinook salmon*. Proceedings of the Royal Society B: Biological Sciences 283: 20160001
3. Parker, G. A. & Pizzari, T. (2010) *Sperm competition and ejaculate economics*. Biological Reviews 85: 897–934
4. Rudolfsen, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. (2006) *Rapid adjustments of sperm characteristics in relation to social status*. Proceedings of the Royal Society B: Biological Sciences 273: 325–332
5. Simmons, L. W. & Fitzpatrick, J. L. (2012) *Sperm wars and the evolution of male fertility*. Reproduction 144: 519–534

Q20: Why Animalia (Metazoans) are more efficient at turning food into usable energy than other life forms

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The driving force of the ATP synthases is a ring of hydrophobic c-subunits embedded in the inner mitochondrial membrane. Their rotation is driven by a transmembrane proton-motive force provided by complexes I, III and IV of the electron transport chain pumping protons out of the mitochondria. Vertebrate and invertebrate c-subunits are extremely conserved.

In the bovine F1-ATPase each rotor-ring consists of eight c-subunits with the N- and C-terminal-helices forming concentric inner and outer rings, with the loop region containing lysine 43 exposed to the phospholipid head-group region on the matrix side of the inner membrane. Trimethylation of this lysine-43 was first discovered in studies of the c subunit stored in various forms of Batten disease. Interactions with cardiolipin allow formation of an insulated ring of only 8 c subunits, in turn allowing ATP synthase to function at a higher gearing than it would if the c ring had more c subunits. Proteomic analysis of c-subunits across representative species from different vertebrates and invertebrate phyla showed that trimethylation of lysine-43 is conserved, so it is likely to be conserved throughout all extant metazoan species. In unicellular eukaryotes and prokaryotes the lysine is unmethylated, and the stoichiometry of c subunits varies from 9–15. Thus metazoan ATP synthases run at a higher gearing than other species allowing more ATP to be made per glucose oxidised. Whereas the mature subunit c sequences are near identical, there are large variations in the 5’ lead sequences which target the nuclear encoded protein to mitochondria. This shows the extreme evolutionary pressure to maintain the c 8 ring stoichiometry in metazoans.

Q21: Screening for epigenetic modifiers and characterising their molecular mechanisms of action, using X inactivation as a model system

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Epigenetic control of gene expression is essential for normal development, and frequently goes wrong in disease. Despite significant efforts over the last decade or more, we still understand relatively little about the molecular mechanisms of epigenetic control, in part because we do not yet know of all of the factors required for these processes. For this reason, my lab has been establishing the molecular tools and cellular systems to perform high throughput screens for novel epigenetic modifiers, or to ascribe new roles to known epigenetic modifiers, using X chromosome inactivation as a model system.

X inactivation has historically been analysed using immunofluorescence based techniques, not amenable to high throughput screening. We have developed fluorescent reporters of expression from the active X chromosome, along with a bespoke shRNA library targeting known or potential epigenetic regulators. Using these tools we perform high throughput screens for epigenetic modifiers and have recently focused on the H3K9 methyltransferase Setdb1, one hit from our screens. We have used allele-specific ChIP-seq, RNA-seq and reduced representation bisulfite sequencing to then characterise how Setdb1-mediated H3K9 methylation contributes to X inactivation. We have found H3K9 methylation is involvednot only in maintaining the inactive state, but also in setting it up when X inactivation is first established. Interestingly, our genomic data shows that H3K9 methylation is enriched on the inactive X at the repetitive regions, yet influences gene silencing and the epigenetic state of the whole chromosome. We propose that silencing of the repetitive elements helps to establish the correct chromosome conformation of the inactive X chromosome, which in turn brings about gene silencing. By continuing to perform similar screens and characterization of hits from these screens, we hope to understand more about how epigenetic silencing occurs at the molecular level.

Q22: Mechanistic and Structure-Function Characterisation of the Epigenetic Regulator SMCHD1

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Structural Maintenance of Chromosomes flexible Hinge Domain-containing 1 (SMCHD1) is an epigenetic regulator that plays critical roles in modulating gene expression. Recently, heterozygous loss of function mutations in *SMCHD1* were identified in facioscapulohumeral muscular dystrophy (FSHD) patients, leading to ectopic expression of the disease-causing gene *DUX4* in muscle cells. While the consequence of SMCHD1 deficiency is well-described, the fundamental question remains as to how SMCHD1 mediates epigenetic control at the molecular level.

We have undertaken a suite of genomics, structural-functional approaches to address this question. We demonstrated that SMCHD1 predominantly binds to regulatory sites in the genome, in part via its C-terminal SMC hinge domain. By performing small-angle X-ray studies, we obtained important insights into the structure and domain organisation of SMCHD1 protein. Moreover, we established that the N-terminal region of SMCHD1 contains a catalytically active GHKL-type ATPase domain, potentially fuel an energy dependent conformational change of SMCHD1 necessary for its engagement with chromatin. We now focus on investigating how pathogenic mutations found in the ATPase domain of SMCHD1 alter its function and how to enhance SMCHD1's ATPase activity as a potential therapy to combat FSHD.

Q23: Fishing for answers with RNA-seq

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Most plants and animals irreversibly differentiate becoming either males or females. However, in some groups, notably fishes, individuals begin life as one sex and reverse sex sometime later (sequential hermaphroditism). Using two distantly related sequential hermaphrodites (NZ spottie and bluehead wrasse) that can be experimentally induced to switch sex, together with transcriptomic analyses and comparative genomic approaches we are working to elucidate the genetic cascade that results in female-to-male sex reversal in fishes. Here I will focus on our experiences with RNA-seq approaches in model (e.g. zebrafish) and non-model systems (wrasses), and in particular the aspects of experimental design that can impact upon the power of a study to find biologically meaningful effects[1] using our studies of sex reversal as a touchstone. I will show that in bluehead wrasse we find that many of the genes classically implicated in sex determination and differentiation in fish and other vertebrates are expressed during sex reversal (e.g. *Dmrt1, Foxl2, Amh*)[2] but their expression patterns are not consistent with roles as the primary triggers of this process. Instead an alternative pathway, Jak-STAT, involved in sex determination in flies but not previously reported to have a role in vertebrate sex determination appears to be closely linked with sex reversal.

1. E.V. Todd, M.A. Black, and N.J. Gemmell (2016). *The power and promise of RNA-seq in ecology and evolution.* Molecular Ecology 25, 1224–1241.
2. H. Liu, M.S. Lamm, K. Rutherford, M.A. Black, J.R. Godwin and N.J. Gemmell (2015). *Large-scale transcriptome sequencing reveals novel expression patterns for key sex-related genes in a sex-changing fish*. Biology of Sex Differences 6, 1-20, (2015).

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
Q24	Characterisation of circular RNA in melanoma cell lines	Bodiyabadu, P.K.[1,2], Sarkar, D.[1,2], Baguley, B.[1], Finlay, G.[1,2], Askarian-Amiri, M.[1,2]	[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, NZ, [2]Deparment of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ
Q25	CpG DNA hypomethylation occurs in human Autosomal Dominant Polycystic Kidney Disease	Bowden, S.[1], Bates, M.[1], Chatterjee, A.[1,2], Rodger, E.J.[1,2], Eccles, M.R.[1,2], Stayner, C.[1]	[1]Department of Pathology, Dunedin School of Medicine, University of Otago, 270 Great King Street, Dunedin 9054, New Zealand. [2]Maurice Wilkins Centre for Molecular Biodiscovery, Level 2, 3A Symonds Street, Auckland 1010, New Zealand.
Q26	Quaternary Structure of lysine biosynthetic enzymes	Cleland, H.S[1], Pearce, F.G[1]	[1]Biomolecular Interactions Centre, University of Canterbury, Christchurch, NZ
Q27	Characterisation and Comparison of β-lactoglobulin Orthologues	Jennifer Crowther[1], Dr Alison Hodgkinson[2], Prof Geoffrey Jameson[3], Assoc. Prof Renwick Dobson[1]	[1]Biomolecular Interaction Centre, and School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [2]Food and Bio-based Products, AgResearch Limited, Ruakura Research Centre, Hamilton, New Zealand, [3]Institute of Fundamental Sciences and the Riddet Institute, Massey University, Palmerston North, New Zealand,

			[4]Bio21 Institute, University of Melbourne, Parkville, Australia
Q28	Characterisation of NagA and NagB from CA-MRSA	Davies J. S.[1], Dobson R.C.J.[1]	[1]Biomolecular Interaction Center, School of Biological Sciences, University of Canterbury, Christchurch, NZ.
Q29	Genomics in New Zealand Forestry	Dungey, H.[1], Telfer, E.[1], Graham, N.[1], Shearer, A.[1,2], Li, Y[1], Klapste, J[1], Murray, M.[1,2], Macdonald, L.[1]	[1] Forest Genetics, Scion, 49 Sala St, Rotorua, New Zealand [2] University of Waikato, Gate 1 Knighton Road, Hamilton 3240, New Zealand
Q30	Expression and Purification of the Adenyltransferase-IMP Cyclohydrolase bifunctional protein of *Candidatus* Liberibacter solanacearum	Gilkes, J.M.[1], Dobson, R.C.J.[1], Frampton, R.[2], Smith, G. [2]	[1]Biological Sciences, University of Canterbury, Christchurch, NZ,[2]The New Zealand Institute for Plant and Food Research, Lincoln, NZ
Q31	The effect of herbicides on the frequency and induction of antibiotic resistance in microbes	Amy Hill[1], Dr Brigitta Kurenbach[1,2], Dr William Godsoe[3], Prof Jack Heinemann[1,2]	[1]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [2]Centre for Integrated Research in Biosafety and Centre for Integrative Ecology, University of Canterbury, Christchurch, New Zealand, [3]Bio-Protection Centre, Lincoln University, Lincoln, New Zealand
Q32	The Escherischia coli Sialoregulon	Chris Horne[1], Renwick Dobson[1, 2]	[1]Biomolecular Interaction Centre, and School of Biological Sciences, University of Canterbury, Christchurch, New Zealand. [2]Bio21

			Institute, University of Melbourne, Parkville, Victoria, Australia.
Q33	Influenza A virus downregulates the expression and induces proteolytic cleavage of antiviral host factor histone deacetylase 6	Mazhar Hussain[1] and Matloob Husain[1]	[1]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand
Q34	Computational characterisation of protein-membrane interactions	William A. Irvine[1], Jack U. Flanagan[2] and Jane R. Allison[1]	[1]Centre for Theoretical Chemistry and Physics, Institute of Natural and Mathematical Sciences, Massey University, Albany, Auckland, NZ, [2]Auckland Cancer Society Research Centre, University of Auckland, Grafton, Auckland, NZ
Q35	*In silico* resolution of the present and past mobilome of *Clostridium difficile* R078 isolates	Jose, B.[1]; Gardner, P.[1]	[1]Biomolecular Interaction Centre, and School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
Q36	The metabolic pathway of volatile sulfur compounds in *Saccharomyces cerevisiae* during wine fermentation	Kinzurik, M. I.[1]; Gardner, R. C.[2]; Fedrizzi, B.[1]	[1]School of Chemical Sciences, University of Auckland, Private bag 92019, Auckland, New Zealand [2]School of Biological Sciences, University of Auckland, Private bag 92019, Auckland, New Zealand
Q37	Characterisation of members of the Dihydrodipicolinate synthase protein sub-family	MacDonald, C.A[1]. Pearce, F.G[2]. Dobson, C.J[3].	[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ, [2,3]Biomolecular Interaction Centre, University of Canterbury, Christchurch, NZ.
Q38	Predicting altered methylation patterns in early pre-eclampsia	Suzan Momani[1,2], Erin	[1]Department of Pathology, Dunedin

		Macaulay[1, 2], Hester Roberts[1, 2], Noelyn Hung[1, 2], Tania Slatter[1, 2], Celia Devenish[3], Ian Morison[1, 2]	School of Medicine, University of Otago, 270 Great King Street, Dunedin 9054, New Zealand [2]Gravida: National Centre for Growth and Development, 2-6 Park Ave, Grafton, Auckland 1142, New Zealand [3] Department of Women's and Children's Health, Obstetrics and Gynaecology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.
Q39	Bacterial sialic acid transport and degradation	Rachel Aimee North[1], Rosmarie Friemann[2], Renwick Charles Joseph Dobson[1]	[1]University Of Canterbury, Christchurch, New Zealand, [2]University of Gothenburg, Gothenburg, Sweden
Q40	Stability and folding of the FOXP3 forkhead domain	Miss. Kershia Perumal[1], Professor Heini Dirr[1], Doctor Sylvia Fanucchi[1]	[1]Protein Structure-function Research Unit, University Of The Witwatersrand, Johannesburg, South Africa, Johannesburg, South Africa
Q41	Experimental evidence that translation initiation in bacteria was invaded by a selfish genetic element	Alannah Rickerby,[1,2] Ryan Catchpole,[1,2,3] Stinus Lindgreen,[1,2] Katherine Donovan,[1,2,] Brigitta Kurenbach,[1] Jack Heinemann,[1] Anthony Poole[1,2]	[1]School of Biological Sciences, [2]Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand, [3]Institut Pasteur, Paris, France

Q42	Magnocellular neuronal activation inresponse to acute myocardial infarction	Roy R.K., Brown, C.H., Schwenke, D.O.	Department of Physiology, University of Otago, Dunedin, New Zealand.
Q43	Many commercial hot-start polymerases demonstrate activity prior to thermal activation	Aaron Stevens[1], Sarah Appleby[1], Martin Kennedy[1]	[1] Gene Structure and Function Laboratory, Department of Pathology, University of Otago, Christchurch, New Zealand
Q44	VEGF-A Release is Higher in Melanoma Cells Harbouring V600E BRaf Mutations	Tran, K.B.[1,3], Kolekar, S.[1], Shih, J.H.[1], Jabed, A.J. [1], Buchanan, C.M.[1,3], Jamieson, S.M. [2,3], Baguley, B.C.[2,3], Shepherd, P.R. [1,2,3]	[1]Department of Molecular Medicine and Pathology, University of Auckland, New Zealand [2]Auckland Cancer Society Research Centre, University of Auckland, New Zealand [3]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand
Q45	Statins as novel therapeutic agents in melanoma	Tran, K.B.[1,3], Kolekar, S.[2], Hunter, F.W. [2,3], Jabed, A.J. [1], Li, D. [2], Shih, J.H.[1], Buchanan, C.M.[1,3], Wilson, W.R. [2,3] , Jamieson, S.M. [2,3], Baguley, B.C.[2,3], Shepherd, P.R. [1,2,3]	[1]Department of Molecular Medicine and Pathology, University of Auckland. [2]Auckland Cancer Society Research Centre, University of Auckland, Auckland. [3]Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland

Poster Abstracts Q24-Q44

Q24: Characterisation of circular RNA in melanoma cell lines

Bodiyabadu, P.K.[1,2], Sarkar, D.[1,2], Baguley, B.[1], Finlay, G.[1,2] , Askarian-Amiri, M.[1,2]

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New Zealand has the highest incidence of melanoma in the world. *ANRIL*, a large non coding RNA is involved in regulating the expression of a number of tumor suppressor proteins in the *CDKN2A/B* locus in human melanoma. This locus houses *p16INK4A* which encodes p16, a potent cell cycle regulator, whose function is lost in nearly 50% of human cancers. *ANRIL* is suggested to interact with the *p16INK4A* locus although the exact nature of their association remains poorly understood. Recent studies have discovered the existence of circular isoforms of *ANRIL* (*circANRIL*) in addition to its linear counterparts. We aimed to investigate the functional mechanism of *ANRIL* by characterising the multiple isoforms of *circANRIL* in melanoma cell lines.

The *circANRIL* were systematically amplified using outward-facing primers designed against each of the exons of *ANRIL*. The resulting PCR products were cloned, sequenced and mapped against the ANRIL genome in order to identify various *circANRIL*. We then localised and quantified *circANRIL* within each sub-cellular fraction by cell fractionation and quantitative-PCR respectively.

Using the results of the sequence analysis we were able to construct a library of all *circANRIL* isoforms identified in melanoma cell lines. The cell fractionation experiments confirmed the cytoplasmic localisation of *circANRIL* which is distinct to the nuclear localisation of its linear counterpart.

The identification of numerous *circANRIL* in melanoma cell lines demonstrates the complexity of the *CDKN2A/B* locus and suggests a complex regulatory network for *p16INK4A* expression. Characterisation of these isoforms enable us to manipulate their expression of further functional analysis. The localisation of *circANRIL* in the cytoplasm, together with previous data, suggests that *circANRIL* may be associating with supramolecular complexes in the cytoplasm and proposes that *circANRIL* may exert its functions as a post-transcriptional regulator of *p16*.

Q25: CpG DNA hypomethylation occurs in human Autosomal Dominant Polycystic Kidney Disease

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The DNA methylation status of the genome has been shown to contribute to development and progression of many cancers. However, the role of DNA methylation in Mendelian diseases has not been extensively studied. The process of cystogenesis in the Mendelian disease Autosomal Dominant Polycystic Kidney Disease (ADPKD) shares similarities with many neoplasms, due to the characteristic large fluid-filled cysts that develop within the kidney. Most ADPKD patients carry a mutation in one copy of the *PKD1* gene, but loss of function of the second *PKD1* allele contributes to disease progression. It has been hypothesised that an epigenetic mechanism such as DNA methylation could contribute to a change in *PKD1* expression in the second allele, playing a role in cyst progression.

To understand the role of DNA methylation in ADPKD, whole genome-scale methylomes interrogating a 30-fold enrichment of the CpG islands of human ADPKD kidney tissue (n=2) were generated using reduced representation bisulfite sequencing, and compared to those of normal kidney tissue (n=3). Comparative data between the two groups indicated overall hypomethylation in the ADPKD tissue, which is similar to the methylation alteration often seen in cancer. There were 25 significantly differentially methylated fragments (DMFs) identified, and 52% of these were hypomethylated. DMFs were identified with an ANOVA test with FDR <0.25 and a 15% difference in methylation status. There was an overall enrichment of DMFs overlapping gene bodies compared to other genomic elements. A hypermethylated DMF was also found within the *PKD1* gene body, overlapping an intron/exon junction. Methylome analysis of additional ADPKD tissue samples, and cell lines derived from individual cysts, will be used to expand these data sets, to develop a rationale for targeting DNA methylation in ADPKD as a suitable therapeutic strategy.

Q26: Quaternary Structure of lysine biosynthetic enzymes

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Dihydrodipicolinate synthase and Dihydrodipicolinate reductase (DHDPS and DHDPR) are two important enzymes in the diaminopimelate (DAP) pathway; a lysine biosynthesis pathway. Lysine is the limiting amino acid in stable grains such as rice and wheat. DHDPS catalyses the first committed step of the pathway and has been a subject of much investigation in bacteria and plants which have different dimeric arreangements[1]. Both bacterial and plant DHDPS typically exist in a tetrameric "dimer of dimers" formation. However the arrangement of the dimers is structurally different. A structural difference is also observed in DHDPR, the enzyme that catalyses the 2nd committed step of the pathway. In bacteria, it exists as a tetrameric enzyme. However, in plants it exists as a dimeric enzyme. Both of these differences are the result of an evolutionary divergence that occurred at some point in the evolutionary lineage; possibly in algal organisms.

The quantification of red and green algal DHDPR structures is underway. Calculation of molecular weights using size exclusion chromatography and analytical ultracentrifugation data will be used to find if the enzymes are more similar to plant dimers or bacterial tetramers. Preliminary analysis of *Chlamydomonas reinhardtii;* a green algal DHDPR has been shown to exist in equilibrium between tetramer and dimer in analytical ultracentrifugation data. *Selaginella moellendorffii* DHDPS is another subject of interest. This lycophyte DHDPS exhibits interesting behaviour as it exists as a dimer with lysine bound but as a tetramer with pyruvate bound. Lysine is an allosteric regulator of DHDPS which appears to cause disassociation the dimeric interface in this particular organism. This effect has been shown in analytical ultracentrifugation experiments under low concentrations of both ligands.

[1]Griffin, M., Billakanti, J., Wason, A., Keller, S., Mertens, H., & Atkinson, S. et al. (2012). *Characterisation of the First Enzymes Committed to Lysine Biosynthesis in Arabidopsis thalian*a.*Plos ONE*, *7*(7), e40318.

Q27: Characterisation and Comparison of β-lactoglobulin Orthologues

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β-lactoglobulin is an abundant whey protein in the milks of ruminant animals yet it is absent in humans. It has been intensively studied using countless biophysical techniques, yet its physiological function remains elusive. Our work focuses on the comparison of the caprine and bovine orthologues of β-lactoglobulin. AUC has proved a useful complement to X-ray crystal structures and small angle X-ray scattering data to provide information on the oligomeric state of these proteins. In both solution and crystalline states they appear to be dimeric. We have also used membrane confined electrophoresis to characterise the effective charge on these molecules, identifying a lesser negative overall charge on caprine β-lactoglobulin which has the potential to affect interactions that occur within milk. A structural understanding of these proteins may provide useful insight into the relationships among the structural, nutritional, immunological and processing characteristics that distinguish cow and goat milk.

Q28: Characterisation of NagA and NagB from CA-MRSA

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Staphylococcus aureus is notorious for its ability to evolve resistance mechanisms against antibiotics. Infections caused by community-associated methicillin-resistant *S. aureus* (CA-MRSA) are now at epidemic level, driving the ever-increasing demand for novel antibiotic development[1]. Recently, *S. aureus* has been shown to utilize sialic acid (*N*-acetylneuraminic acid) as a carbon source *in vitro*[2]. This ability is hypothesized to afford *S. aureus* a competitive advantage in colonization[2], and the enzymes involved could therefore be considered potential drug targets. NagA and NagB catalyse the final steps of this utilization pathway, and are yet to be structurally characterised. NagA catalyses the deacetylation of *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) to glucosamine-6-phosphate (GlcN-6-P), a precursor for peptidoglycan biosynthesis. NagB catalyses the deamination of GlcN-6-P to the glycolysis intermediate fructose-6-phosphate (Fru-6-P). Alongside GlmS (Fru-6-P amidotransferase), NagA and NagB are thought to form a 'gate' controlling the distribution of sugars to these important pathways, whose regulation is not fully understood[3].

CA-MRSA NagA and NagB were cloned into pET-30ΔSE expression vectors, verified by sequencing, then transformed into *E. coli* BL21 (DE3) for protein overexpression. Purification of both was achieved via anion-exchange, hydrophobic and size-exclusion chromatography. Both proteins were then characterised using differential scanning fluorimetry (DSF), analytical ultracentrifugation (AUC) and small-angle X-ray scattering (SAXS). The SAXS and AUC data show both NagA and NagB exist primarily as dimers in solution. Dimeric NagB has not been observed in other gram-positive bacteria, and may point to differences in function and regulation. Low resolution models have been constructed *ab initio* using the SAXS data. Multiple sequence alignment and SDS-PAGE analysis together suggest that the NagB dimer may be held together by disulfides, similar to the *E. coli* NagB trimer interface[4]. Crystallization trials have yielded crystals of both NagA and NagB that gave low-resolution diffraction data, with these crystallization conditions currently being optimized.

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2. Olson, M. E., King, J. M., Yahr, T. L., and Horswill, A. R. (2013) *Sialic acid catabolism in Staphylococcus aureus*. J Bacteriol **195**, 1779-1788
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4. Oliva. (1995) *Structure and catalytic mechanism of glucosamine 6-phosphate deaminase from Escherichia coli at 2.1 A resolution*. Structure

Q29: Genomics in New Zealand Forestry

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Tree breeding in New Zealand has initiated the move to genomics. Tree breeding programmes in NZ are currently all with exotic species, with recorded pedigree depths of between two and four generations from unimproved. The combination of well-structured populations and the ability to clonally test across a number of environments is an advantage in these programmes. However, the limited number of generations means that these populations are still highly heterogeneous. Furthermore, most experimental trials are planted with wind-pollinated trees and have long generation times, both of which present a significant challenge to breeding programmes.

Genomics can help address many of the challenges faced by tree breeders: providing marker-based relationships in wind-pollinated populations where only one parent is known, selecting for wood quality traits that are very expensive to measure, and accelerating the rate of delivery of genetic gain. The Radiata Pine Breeding Company, in partnership with Scion, is leading genomics research for radiata pine, and have developed a 44K exome capture panel to enable the implementation of genomic selection. The development of such extensive genomics resources has historically been unachievable for smaller breeding programmes, such as those for eucalypts and Douglas-fir. However, recent years have seen internationally-developed high density SNP arrays become available for these species [1, 2], which is enabling a paradigm shift in our approach to breeding.

We report on the progress being made towards the introduction of genomics and genomics research in radiata pine, Douglas-fir and in a number of eucalypt breeding programmes. We present early results from the existing multi-species *Eucalyptus* SNP chip EuCHIP60K that has been used in the first application of genomics to a eucalypt breeding programme in NZ. We also report on our plans to effectively incorporate genomics for a number of other forest-based species.

1. Howe, G.T., et al., *A SNP resource for Douglas-fir: De novo transcriptome assembly and SNP detection and validation.* BMC Genomics, 2013: p. 137.
2. Silva-Junior, O.B., D.A. Faria, and D. Grattapaglia, *A flexible multi-species genome-wide 60K SNP chip developed from pooled resequencing of 240 Eucalyptus tree genomes across 12 species.* New Phytol, 2015. **206**(4): p. 1527-40.

Q30: Expression and Purification of the Adenyltransferase-IMP Cyclohydrolase bifunctional protein of *Candidatus* Liberibacter solanacearum

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Zebra chip disease (ZC) is putatively caused by the alphaproteobacteria, "*Candidatus* Liberibacter solanacearum" (Lso) which is vectored by the tomato/potato psyllid. The disease results in the development of blacklines in fried potato tubers. Due to the devastating effect of CLso, measures to thoroughly understand the biology and aetiology of CLso have been undertaken resulting in the complete genome sequence of Lso in 2011. The completion of the Lso genome has provided insight into the metabolic processes and genes of Lso. A unique feature of the Lso genome is the presence of a number of putative bifunctional proteins. As Lso has a characteristic small genome, it is expected that it would utilize the host organisms processes to replace the lost functions with the result being a more simplified genome, contrastingly, bifunctionals increase genome complexity therefore raising the question as to why these bifunctionals have arisen over mono-functionals. Many protein functions can be inferred from the known functions of homologous proteins, but the existence of bifunctionals complicates this and provides a window into the complex reactions that compose the organism. With the availability of sequence data, the next step is to determine the functions of the encoded proteins.

Lso contains the bifunctional enzyme adenyltransferase/IMP cyclohydrolase (ATIC) which catalyses the last two steps in the purine biosynthetic pathway: the conversion of aminoimidazole-4-carboxamide ribonucleotide (AICAR) to the final product IMP. It has been shown that de novo purine synthesis is required for optimal virulence in *S. aureus* and *X. oryzae*, therefore highlighting the potential for ATIC to be a potential antibacterial target. The ATIC bifunctional protein (59kDa) of CLso was expressed in *E. coli* BL21DE3 cells. The protein was purified using anion exchange, hydrophobic interaction, size exclusion and Q-sepharose strong anion exchange chromatography. With the purification of CLso ATIC, further structural, kinetic and inhibition studies can be carried out to elucidate the potential as an antibacterial target.

Q31: The effect of herbicides on the frequency and induction of antibiotic resistance in microbes

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Antimicrobial resistance is estimated to kill 700,000 people annually. By 2050 this number could rise to 10 million unless resistance is better managed. A more complete understanding of how resistance develops and is maintained is needed to avoid a future where treatable infections again become lethal challenges. We have been investigating the effects of chemicals released in high amounts, such as herbicides, on the antibiotic resistance of Escherichia coli and Salmonella enterica serovar Typhimurium. Increases and decreases in antibiotic susceptibility were seen when bacteria were exposed to both herbicide and antibiotic, compared to the antibiotic alone. These changes were reversible and due to changes in gene expression patterns. We hypothesized that these reversible changes could lead to permanent increases in antibiotic resistance.

(A) A herbicide-induced increase in antibiotic resistance allows bacteria to survive for more generations in the presence of the antibiotic, increasing the chance of permanently resistant mutants arising. We determined the mutation frequency (number of mutants/total number of bacteria) of a bacterial culture after exposure to herbicide and antibiotic, herbicide only, or no treatment for ~20 generations. Depending on the combination tested, we found 103 to 106-fold higher mutation frequencies in the herbicide and antibiotic treatment compared to the controls. (B) We hypothesized that a herbicide-induced increase in antibiotic susceptibility could cause selection in favour of resistant bacteria at lower concentrations of antibiotic. We competed two strains with different antibiotic resistance levels in a range of sub-inhibitory antibiotic concentrations. The herbicide and antibiotic treatment caused significant selection in favour of the more resistant strain. The antibiotic/herbicide alone caused no selection or selection in favour of the more susceptible strains at the chosen concentrations. This work provides evidence that the chemicals bacteria may be exposed to in their natural environment could create conditions that select and mantain antibiotic resistance.

Q32: The *Escherischia coli* Sialoregulon

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Sialic acids are one of the most important amino sugars in biology. Found at the terminal end of glycan molecules, these negatively charged sugars act as receptors for cellular communication. Sialic acids also serve as a carbon, nitrogen, and energy source for bacterial pathogens that degrade it into fructose-6-phosphate, which can subsequently enter central metabolism. The canonical pathway that breaks down sialic acid following its import into the cell includes a lyase, kinase, epimerase, deacetylase, and a deaminase. This pathway is regulated in bacteria by the *nan*Repressor, however detailed structural evidence explaining this process is lacking. The *nan*Repressor from *Escherichia coli* has been experimentally shown to regulate the *nan*CMS and *yjh*BC operons, in addition to the canonical degradation pathway by binding to two or three tandem base pair repeats of the DNA sequence GGTATA. Collectively, the nan genes that are directly controlled by the *nan*Repressor are designated as being part of the 'Sialoregulon'. While the biological role of the *nan*CMS operon is currently being studied, the biological role of the *yjh*BC operon has yet to be established. The primary function of this operon is believed to collectively provide the ability to convert and utilise less common derivatives of sialic acid to *N*-acetylneuraminic acid; the preferred substrate for the lyase in the degradation pathway. Our overall aim is to develop the first detailed 'picture' of how the *nan*Repressor regulates gene expression and elucidate the biological role of the enzymes in the wider pathway.

Q33: Influenza A virus downregulates the expression and induces proteolytic cleavage of antiviral host factor histone deacetylase 6

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Influenza A virus (IAV) is a successful human respiratory pathogen causing ~1 billion cases of flu and 300,000 to 500,000 deaths worldwide annually. Influenza has prevented the development of a universal vaccine and rendered approved antiviral drugs almost ineffective. A comprehensive understanding of IAV-host interactions is needed to develop effective and long-lasting anti-IAV strategies. Our lab has discovered that histone deacetylase 6 (HDAC6) is a novel anti-IAV host factor that restricts IAV infection by downregulating the trafficking of viral components to the site of virus assembly [1]. Viruses are known to antagonize antiviral host factors to efficiently replicate. During my PhD, I am studying that how IAV antagonizes the anti-IAV function of HDAC6. So far, I have found that IAV downregulates the HDAC6 expression both at mRNA (>85% reduction after 24 hours) and polypeptide (~75% decrease after 24 hours) level in human lung epithelial cells. Furthermore, HDAC6 polypeptide is also being cleaved at multiple positions during the course of IAV infection. Treatment of infected cells with inhibitors of the lysosomal and proteasomal degradation pathways as well as apoptotic pathway indicate that HDAC6 polypeptide is sequentially degraded by lysosomes-induced apoptotic pathway. Next, I will identify the lysosomal protease(s) responsible for inducing this pathway. I will also identify the cleavage sites in HDAC6 polypeptide and determine the significance of HDAC6 degradation in IAV infection.

1. Husain M, Cheung CY. (2014). *Histone deacetylase 6 inhibits influenza A virus release by downregulating the trafficking of viral components to the plasma membrane via its substrate, acetylated microtubules.* Journal of virology. 88(19):11229-11239.

Q34: Computational characterisation of protein-membrane interactions

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As much as 30% of the human genome encodes membrane proteins, many of which act as drug targets whether they be signalling proteins or enzymes. Characterising the behaviour of these proteins more often than not requires an understanding of the interaction between the protein and the cellular membrane interface.

This research uses a series of molecular dynamics and analytical techniques to pinpoint the driving factors behind the PI3K enzyme's interactions with a bilayer representative of a brain cellular membrane.

Q35: *In silico* resolution of the present and past mobilome of *Clostridium difficile* R078 isolates

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The genome of the human pathogen *Clostridium difficile* contains many mobile genetic elements (MGEs) which contribute to its virulence, and resultant repeat-rich regions makes it difficult to resolve these elements with short read NGS technologies. New assembly tools and SMRT-sequenced genomes of two R078 *C. difficile* strains were used to resolve difficult sequence regions and create an improved draft genome of a *C. difficile* estuarine isolate.

CRISPRTarget was used to identify protein targets of the CRISPR/cas systems found in the isolates to determine resistance to bacteriophage infection and may be useful for identifying potential phage evolution, predicting the existence of novel phages and establishing a potential host range of existing uncharacterised phages.

Q36: The metabolic pathway of volatile sulfur compounds in *Saccharomyces cerevisiae* during wine fermentation

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Volatile sulfur compounds (VSCs) are important contributors to the distinctive aroma of wine, cheese and other fermented foods, leading to either consumer acceptance or rejection. In the model yeast *Saccharomyces cerevisiae*, there has been a significant amount of work on elucidating the sulfate assimilation pathway leading to the production of sulfurylated amino acids, yet not a lot is known about how VSCs are produced in wine. There are different hypothesis regarding the nature of the sulfur-containing precursors for VSCs in wine with L-methionine, L-cysteine and sulfate being the main candidates. Fermentation of synthetic grape media with either ^{13}C- and ^{15}N -labeled L-methionine or L-cysteine or ^{34}S sulfate revealed distinct catabolic routes into volatiles. One route involves the incorporation of hydrogen sulfide into heavier onion-off flavour VSCs. A second route apparently de-aminates but preserves the main carbon skeleton of L-methionine, releasing methanethiol. Further insights into the pathways are discussed.

Q37: Characterisation of members of the Dihydrodipicolinate synthase protein sub-family

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Members of the Dihydrodipicolinate synthase (DHDPS) sub family have similar structural configurations while having diverse functions. Some are well characterised while others are less studied when compared to DHDPS. This project aims to characterise some of these proteins in order to further understand their evolutionary relationships. This project investigated Trans-hydroxybenzylidenepyruvate hydratase-Aldolase (t-HBP-HA), a member of this sub family, from Pseudomonas fluorescens which catalyses the final step of the naphthalene dehydrogenase pathway. 1-pyrroline-4-hydroxy-2-carboxylate deaminase (HypD) is also a member of this sub-family and is investigated here. This project used Analytical ultracentrifugation (AUC) and x-ray crystallography to probe the structural characteristics of these proteins.

T-HBP-HA proteins have been previously studied in past projects[1], with past gel results suggested a trimeric orientation. AUC was used to determine the oligomeric state of this protein. The results for t-HBP-HA showed a sedimentation value of 7.85S which is consistent with tetrameric proteins, not trimeric. It was crystalized and its structure solved to 2.2 Armstrong. This is the only t-HBP-HA protein that has currently been crystallised. Its oligomeric configuration was shown to be a tetramer, which is consistent with previous AUC results. The quaternary arrangement is also consistent with bacterial DHDPS. AUC was used to determine the different oligomeric states of the HypD proteins. Four HypD proteins from; Agrobacterium tumefaciens, Sinorhizobium meliloti, Brucella melitensis and Microvirga lupini were investigated and the sedimentation values 9.2S, 9.45S, 9.6S and 10.32S respectively. These values are consistent with hexametric proteins. The oligomeric state of t-HBP-HA is confirmed to be a tetramer as opposed to a trimer, this is significant due to there currently being no trimeric DHDPS proteins. HypD being a hexamer is significant, again, to members of the DHDPS sub-family being mostly tetramers.

Q38: Predicting altered methylation patterns in early pre-eclampsia

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Preeclampsia is one of the most common adverse pregnancy outcomes that complicates about 5-10% of pregnancies worldwide, but the underlying causes are mostly unknown. The identification of biomarkers that could be used to accurately identify those women at increase risk for the later development of pre-eclampsia would be a major step forward in antenatal care. Our lab has been used reduced-representation bisulfite sequencing (RRBS) in order to compare epigenetic methylation differences between dysfunctional pre-eclamptic placentas and matched controls. A huge dataset of differential methylation between the pre-eclamptic and control placentas is already provided from our group and validation studies have been carried out and further validation analysis will be carried on.

Also, the existence of circulating cell-free DNA (ccf-DNA) isolated from maternal blood plasma could allow for the detection of these differences from the early stage of pregnancy. As a part of my PhD project, I will differentiate between maternal DNA and cff-DNA using some epigenetic markers that have shown opposite and extreme differences in methylation. I'm currently using deep sequencing of PCR products performing MiSeq platform to target our candidate regions for pre-eclampsia. I ultimately hope to identify a DNA methylation signature of pre-eclampsia in maternal blood plasma that can be used clinically to predict women who are at risk of developing this threatening condition of pregnancy.

Q39: Bacterial sialic acid transport and degradation

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In heavily sialylated environments, bacterial pathogens import and degrade host-derived sialic acid as a nutrient source and this pathway constitutes a novel and unexploited target for antibiotic drug design. Three sialic acid transporters, from two distinct gene families, were investigated to probe how sialic acid is transported across the cytoplasmic membrane. The oligomeric structures of these transporters were investigated using analytical ultracentrifugation. Excitingly, the first crystal structure of a sialic acid transporter will be presented. It was solved in complex with sialic acid and two sodium ions, providing insight into how this transporter mediates the movement of sialic acid across the membrane. Overall, this work provides new data that enriches our understanding of the import and degradation of sialic acid in clinically important human bacterial pathogens.

Q40: Stability and folding of the FOXP3 forkhead domain

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The FOXP (FOXP1-4) subfamily of transcription factors is unique in that their DNA-binding domains (forkhead domains) exhibit domain swapping where structural elements are exchanged via extension of the hinge-loop region. FOXP3 is expressed solely by $CD4^+$ T cells and functions to suppress proliferation of effector T cells to maintain immune system homeostasis. The FOXP3 DSD is shown to be critical for its suppressive function. Furthermore, mutations in the DSD are linked to a severe autoimmune disease known as IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). The aim of this study is to obtain the complete folding pathway of the FOXP3 DSD in order to shed more light on how FOXP3 functions at a molecular level. In the absence of DNA, circular dichroism and fluorescence spectroscopy both indicate that the protein is partially unfolded. Electrophoretic mobility shift assays indicate that the DSD binds DNA and is indeed functional. In addition, the urea-induced equilibrium unfolding pathway shows non-cooperativity and lacks a pre- and post-transition region which is characteristic of intrinsically disordered proteins. Upon binding to DNA, the secondary structure does not change significantly, however the tertiary structure becomes more compact and indicates burial of tryptophan residues compared to that of unbound protein. This data suggests that the FOXP3 DSD is intrinsically disordered in the absence of DNA and becomes more structured upon DNA binding. Further studies including equilibrium unfolding in the presence of DNA as well as unfolding and refolding kinetics will be performed in order to elucidate the complete folding and unfolding pathways of the FOXP3 DSD.

Q41: Experimental evidence that translation initiation in bacteria was invaded by a selfish genetic element

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The core machinery for protein synthesis is universal to cellular life. However, idiosyncrasies exist that differentiate the process of translation across the three domains (Archaea, Bacteria, Eukarya). One such example is found in bacteria, mitochondria and chloroplasts, where a formyl group is added to methionine prior to initiation of translation. Intriguingly, this formyl group is removed from the nascent polypeptide by peptide deformylase before protein production is complete, and appears to have no clear function. Despite this, it is essential to bacterial translation: interrupting formylation is deleterious. A well conserved process that, if lost, leads to a severe phenotype is usually associated with important function. However, previous work in our group indicates that formylation, and the removal of the formyl group by deformylation, likely evolved from an ancient, plasmid-transmitted, toxin-antitoxin system capable of post-segregational killing. Post-segregational killing systems get their name because of how they act: the antitoxin is more labile than the toxin, so segregating daughter cells that do not inherit the toxin-antitoxin gene pair die through action of the long-lived toxin. This creates an 'addiction' to the system, because cells can gain the genes, but cannot subsequently lose them. We predicted that the formyl group is toxic to the cell, and that subsequent removal by peptide deformylase negates this toxicity. A line devoid of the formylase and deformylase genes was produced. While initially very unfit, after 1,500 generations of evolution, we found that growth rates of knockout lines were identical to the wild-type lineages. We showed that there were mutational changes to genes involved in translation which enabled the cells to adapt to the loss of formylation. Moreover, introducing the genes on a plasmid elicited a post-segregational killing phenotype, as per our model. To reproduce the initial process of adaptation to this element, we reintroduced the genes on the chromosome of *E. coli*, and subjected eleven lines to a further 3,000-generation evolution experiment. Our results indicate that, despite our initial lines not requiring formylation, addiction to the gene-pair has reasserted itself, with the genes appearing essential. Our results thus suggest that bacterial formylation invaded and spread through this domain via addiction, with these genes becoming 'essential' as a result of their capacity to elicit post-segregational killing.

Q42: Magnocellular neuronal activation in response to acute myocardial infarction

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Acute myocardial infarction (MI) is a global health problem, which is associated with the alteration of the neuro-hormonal homeostasis. Hormones that are elevated following acute MI are also produced by the hypothalamic magnocellular neurons. However, the activation level of these magnocellular neurons is yet to be explored.

In this study we aimed to assess the activation of magnocellular neurons in response to acute MI. Magnocellular neurons were located in two hypothalamic nuclei; supraoptic nucleus (SON) and paraventricular nucleus (PVN). In order to investigate their activation, rats were transcardially perfused under anesthesia 90 min following acute MI or sham operation. Immunohistochemistry for Fos protein was performed on brain sections as a marker of neuronal activation. MI rats had a significantly higher number of Fos-positive cells in the SON and PVN than sham operated rats (p=0.0002, unpaired t-test and p<0.0001, unpaired t-test respectively).

We next determined the phenotype of the activated magnocellular neurons using double label immunohistochemistry. In SON, significantly higher number of Fos-positive oxytocin (OT) neurons was identified compared to sham (p<0.0001, unpaired t-test). In PVN, acute MI was associated with significantly higher number of Fos-positive vasopressin (VP) and oxytocin (OT) neuron to sham (p=0.0022, unpaired t-test and p<0.0001, unpaired t-test respectively). Taken together these results suggest that the activation of magnocellular VP neurons may co-relate to the increased expression of circulating VP hormone, which has been previously reported in chronic heart failure.

Q43: Many commercial hot-start polymerases demonstrate activity prior to thermal activation

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Generation of non-specific products and primer-dimers during polymerase chain reaction are significant and common hinderances to successful DNA amplification. The development of hot-start DNA polymerases that become active only once heated above primer annealing temperatures, was a major advance, and these enzymes are now widely used. However, we have discovered that a surprising proportion of commercially available hot-start polymerase preparations have activity prior to heat activation, which may lead to poor results. The primer-dimer formation assay we describe here is a simple and effective means for testing hot-start polymerase enzymes prior to their use in critical applications.

Q44: VEGF-A Release is Higher in Melanoma Cells Harbouring V600E BRaf Mutations

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Background VEGF-A is an essential mediator in tumour microenvironment for cancer progression and metastasis. Therefore, targeting its signalling pathway has been an important approach in a number of cancers. However, the link between VEGF-A biology and the highly diverse genotypes of melanomas remains unclear.

Aims To study the link between VEGF-A release and melanoma mutations and how this information might be used to improve the current treatment.

Methods Melanoma cells were extracted from patients' biopsies and cultured under a physiologic 5% O_2 environment. Genetic analysis was performed in 102 melanoma cell lines. VEGF secretion was measured by Milliplex kits. VEGF-A and VEGFR2 genes were knockout using CRISPR/Cas9 and inhibitors. Antitumorigenicity was studied in in vitro and in vivo using immunodeficient mice.

Results Our melanoma line panel is representative of the spectrum of human melanoma as it has percentages of mutations similar to those previously described in melanoma tumours. Our most important observation was that VEGF-A secretion levels were significantly higher in melanoma cells harbouring BRAF/V600E mutation than wild type BRAF cells. Furthermore, vemurafenib upregulated VEGF-A secretion in RAS-mutant cell lines. This lead us to investigate the effects of vemurafenib in wild type BRAF xenografts. Strikingly, we observed that the RAS-mutant NZM40 xenograft grows faster with vemurafenib and that the VEGFR2 inhibitor axitinib has very little effect alone; but when vemurafenib and axitinib are combined, the combination has a very strong synergistic effect in inhibiting tumour growth.

Discussion/Conclusion Our study reports for the first time that melanoma cells containing V600E mutation have higher levels of VEGF-A secretion than other cells. In addition, our data also shows evidence about the link between VEGF-A secretion and vemurafenib-induced paradoxical growth of wild type BRAF tumours. Importantly, our data suggest that the efficacy and therapeutic utility for BRAF inhibitors might be significantly expanded by combination therapy with a VEGFR2 inhibitor.

Q45: Statins as novel therapeutic agents in melanoma

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Background HMG CoA reductase inhibitors, better known as statins, were previously reported to have anticancer effects in a number of cancer types. However, the roles and mechanisms of statins in melanoma remains unclear.

Aims To study effects and mechanisms of statins in melanoma and how this information might be utilised to improve the current treatment outcome for melanoma patients.

Methods Melanoma cells extracted from patients were treated with statins and analyzed for proliferation, invasion, apoptosis, autophagy, and cell cycle progression. Antitumorigenicity was studied in vivo using mice models. A novel approach using Genome-scale CRISPR/Cas9 Knockout was performed to study gene-related mechanisms.

Results Data obtained in 45 melanoma cell lines demonstrated statins inhibited the growth of melanoma cells at potentially therapeutic windows across all genotypes. The effect could also be observed with other inhibitors of the mevalonate pathways. Interestingly, RAS mutant cell lines were not more sensitive to statins as earlier reported. Statins also induced apoptosis and autophagy, arrested cell cycle at G0/G1 phase, and inhibited cell migration. In xenograft model, simvastatin at all doses tested (ranging from 100 – 800 mg/kg) delayed NZM37 tumour growth. Furthermore, statins potentiated the efficacy of a number of targeted therapies in synergistic or additive manners. Importantly, we have successfully established statin-resistant clones of melanoma cells after being transduced with a sgRNAs library targeting all genes in the genome and deep sequencing results will be presented.

Discussion/Conclusion Our results suggest that statins at clinically relevant concentrations have strong antitumorigenicity against melanoma cells. Significantly, our data are from the largest panel to date of a broad genotype spectrum of melanoma cell lines, which allows the comparison between mutation groups and for the first time gene-relating mechanisms of statin anticancer effects will be presented.