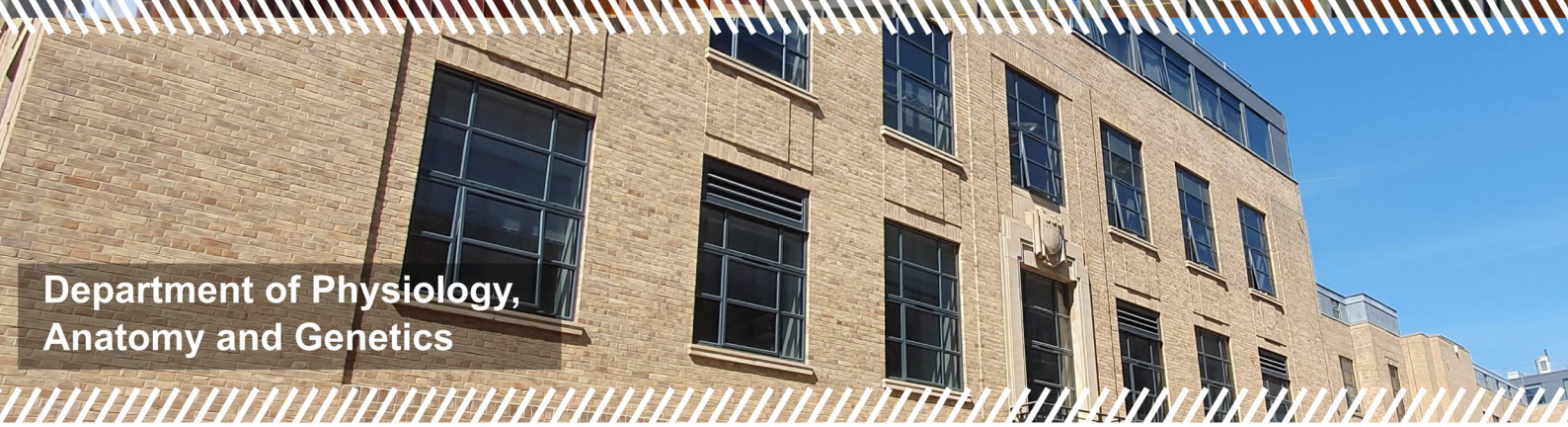
UNIVERSITY OF OXFORD

Interdepartmental Postdoctoral Poster Session

Thursday 16 May 2019

Sir William Dunn School of Pathology

Department of Biochemistry



Department of Physiology, Anatomy and Genetics

Program

14:00 Poster Session opens
Odd numbered posters
Presenters will be required to stand by their posters

15:15 Even numbered posters
Presenters will be required to stand by their posters

16:30 Prize announcement followed by wine reception

17:30 Poster Session ends

Event will be followed by a dinner at the University Club

Poster – 01

Surviving metabolism: acidity as a selection pressure in colorectal cancer cell lines
by Johanna Michl

Introduction: Extracellular acidity is a chemical signature of tumours. It arises because the raised metabolic rate in cancer cells releases large quantities of lactic acid and CO2 into the tumour microenvironment (1, 2). Dysregulated pH has been shown to perturb or even kill cancer cells. Although targeting acidity is a good candidate for the therapeutic management of tumour growth (3), so far, none of the major approved therapies are based explicitly on disrupting acid handling and/or signalling. Using a large panel of colorectal cancer (CRC) cell lines, our aim was to identify the molecular processes that provide a survival benefit to cancer cells living under acid-stress. Methods: We investigated pH-related physiology in over 40 CRC cell lines. We measured (i) survival by sulphorhodamine B (SRB) assay, (ii) lumen formation by F-actin staining, (iii) extracellular pH (pHe) time courses as a readout of metabolic rate, and (iv) intracellular pH (pHi) on a single-cell level by fluorescence imaging. These measurements we performed over a range of pHe values using an imaging plate reader (Cytation 5, Biotek). Results: CRC cell lines displayed a wide range of sensitivities to changes in pHe. A 50% reduction in survival was associated with pHe values of 7.3 (most sensitive: Iscerol) to below 6.5 (least sensitive: C106). pHe-sensitivity was also observed in 3D cultures grown in Matrigel. CRC cell lines categorised as pH-sensitive formed smaller colonies under acidic pHe (6.76); however, their differentiation status was independent of pHe. Furthermore, the cell lines differed in their ability to regulate pHi in response to changes in ambient pHe, quantified in terms of the slope of their pHi-pHe relationship. This metric of pHi-control ranged from 0.16 (Colo206) to 0.59 (Iscerol), representing strong to weak pHi control, respectively.

Conclusions: We established a database that provides a comprehensive appraisal of the pH phenotype for individual CRC cell lines. This information allows us to correlate phenotype with mutations and gene expression profiles of these cell lines, and to identify molecular processes involved in acid handling and signalling.

References:
1. Parks, S.K., J. Chiche, and J. Pouyssegur, Disrupting proton dynamics and energy metabolism for cancer therapy. Nat Rev Cancer, 2013. 13(9): p. 611-623.
2. Swietach, P., R.D. Vaughan-Jones, and A.L. Harris, Regulation of tumor pH and the role of carbonic anhydrase 9. Cancer and Metastasis Reviews, 2007. 26(2): p. 299-310.
3. Neri, D. and C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov, 2011. 10(10): p. 767-7

Poster – 02

How Nanoscale Protein Interactions Determine the Mesoscale Dynamic Organisation of Membrane Proteins
by Anna Duncan

The spatiotemporal organization of membrane proteins is often characterised by the formation of large protein clusters and complex mixtures of lipids. Modelling the nanoscale heterogeneities within physiologcial membranes in molecular detail, has been confounded by the inherent difficulties of simulating large numbers of proteins over meaningful timescales. We have developed and a mesoscale model that can incorporate 1000s of proteins, trained on the results of coarse-grained molecular dynamics simulations1. We achieve simulations over timescales that allow direct comparison to experimental data, in particular, the single molecular tracking of bacterial outer membrane proteins (OMPs). In Escherichia coli, OMP clustering leads to OMP islands, the formation of which underpins OMP turnover2 and drives organization across the cell envelope3. Using molecular dynamics and mesoscale simulations we show that specific interaction surfaces between OMPs are key to the formation of OMP clusters, that OMP clusters present a mesh of moving barriers that confine newly inserted proteins within islands, and that mesoscale simulations recapitulate the restricted diffusion characteristics of OMPs in vitro. We extend the mesoscale model to explore the impact of lipid composition and protein activity on the organisation of receptors in mammalian cell membranes. The mesoscale model provides a powerful integrated approach to understand physiologically accurate biological membranes at mesoscale length and time scales.

Poster – 03

Screening colorectal cancer cell lines for connexin-dependent intercellular diffusive coupling
by Stefania Monterisi

According to a widely held canon, most types of cancer have little or no cell-to-cell diffusive coupling involving gap junctions (GJs). Moreover, connexin (Cx) proteins that assemble GJs, have been considered for long time as tumour suppressors, putatively because they would restrict cancer cell motility and limit the extent to which a cancer cell can regulate its cytoplasmic milieu independently of neighbouring cells. Recently, we have shown that some cancer cell lines such as pancreatic ductal adenocarcinoma Colo357, are diffusively coupled by Cx43 channels, which establishes a pathway for exchanging metabolites, including the metabolic end-products lactate and H+ ions (3). Here, we screened a panel of colorectal cancer (CRC) cell lines for evidence of coupling in monolayers.

A gene expression database from over 100 CRC cell lines was searched for expression levels of connexin-coding genes. Further measurements of cell-to-cell coupling were performed on a selection of CRC cell lines that include various combinations of Cx isoform expression, ranging from high to low or absent. The protein products were tested by western blot and immunofluorescence. Diffusive coupling was quantified in calcein-loaded monolayers by fluorescence recovery after photobleaching (FRAP), which involves bleaching one cell in a monolayer and measuring the time constant of fluorescence recovery, which can then be converted to a permeability constant.

Our results show that cell-to-cell connectivity varies between CRC cells and even within subpopulations of a CRC line. The extent of diffusive coupling between connected cells is sufficient to permit a substantial flux of small molecules, including metabolites. This could be particularly important in solid tumours where large diffusion gradients of metabolites, forming along gradients of oxygen, can drive net fluxes through coupled cells and hence influence tissue physiology.

Poster – 04

Prediction of protein-lipid binding energies using free energy calculations
by Robin Corey

Many integral membrane proteins are structurally and/or functionally regulated by specific interactions with membrane lipids. The structural basis of these interactions can be resolved to some degree with a number of experimental and computational approaches. The energetic basis of these interactions, however, has proven more difficult to resolve, with molecular simulation the primary technique employed in this regard. These simulations typically prove both computationally expensive and to carry out and difficult to produce reliable data from. Here, we build on previous work modelling the energetics of protein-lipid interactions using potential of mean force calculations, extending the analyses to incorporate two additional free energy based simulation approaches: alchemical free energy perturbation (this poster) and well-tempered metadynamics (see poster of Owen Vickery). We adapt these techniques for use with a coarse-grained force field, and apply them to a number of different protein-lipid systems. We find a good agreement between the different techniques, and provide a solid framework for easy implementation of the technique by other groups.

Poster – 05

System-wide profiling of protein-RNA interactions uncovers host regulators of virus infection
by Manuel Garcia-Moreno

RNA is a central molecule in virus biology, yet viral genomes only encode a few proteins able to interact with RNA. Hence, viruses rely on host RNA-binding proteins (RBPs) to accomplish their biological cycle. RBPs are also key components of the antiviral response, sensing intermediaries of viral replication. However, the complement of host RBPs involved in virus infection has remained largely unknown.

To address this question, we developed “comparative RNA interactome capture” and applied it to human cells challenged with a model RNA virus, called sindbis (SINV). Briefly, mock and infected cells are irradiated with ultraviolet light to crosslink protein and RNA interacting directly. Polyadenylated (viral and host) RNA is purified via oligo (dT) capture and

the associated RBPs are identified by quantitative proteomics. Our data reveal that SINV affects the RNA-binding activity of over two hundred proteins. In particular, SINV shuts down key regulators of nuclear RNA metabolism, while it activates RNA degradation, protein synthesis and antiviral factors. Regulation of RBP activity is largely explained by the loss of cellular mRNAs while viral RNA accumulates. Strikingly, most virus-induced RBPs concentrate in the viral replication factories and control the capacity of the virus to infect the cell. For example, cells lacking XRN1 are refractory to SINV infection, while GEMIN5 impairs viral gene expression. Cellular RBPs thus represent promising targets for future antiviral therapies.

Poster – 06

Molecular modeling and simulations of the E. coli serine receptor and chemosensory complex
by Keith Cassidy

In Escherichia coli, transmembrane chemoreceptors couple environmental chemical gradients to the activity of an associated histidine kinase and ultimately cellular swimming pattern, enabling a behavior known as chemotaxis. Chemoreceptors transduce sensory signals though several adjacently-coupled signaling elements whose structure and dynamics are affected by ligand binding and reversible methylation. Though the subject of several decades of study, the detailed molecular mechanism of chemoreceptor signal transduction remains unresolved. Here, we present an atomistic model of the complete membrane-bound E. coli serine receptor (Tsr), derived from existing structural data and molecular simulations. Utilizing microsecond-timescale molecular dynamics simulations, we investigate transmembrane signaling and methylation-based adaption in Tsr, revealing residue-level insights into both processes. Additionally, combining our Tsr model with new, highly-resolved cryo-electron microscopy data, we construct a model of the complete E. coli core signaling unit, the minimal complex required for basic receptor-mediated kinase regulation.

Poster – 07

To catch a thief: Finding the RNA binding proteins hijacked by HIV-1
by Marko Noerenberg

Due to their limited coding capacity, most viruses rely on the host machinery to accomplish their biological cycle. In particular, the human immunodeficiency virus type 1 (HIV-1) requires several host RNA-binding proteins (RBPs), to transcribe, translate and encapsidate its RNA genome.

In addition, RBPs play important roles as sensors of intermediaries of viral replication in the antiviral response. However, the complete repertoire of cellular RBPs utilised by HIV-1 remains largely unknown.

To identify RBPs playing a role in HIV-1 biological cycle, we applied here RNA interactome capture to a CD4+ T-cell line infected with HIV-1. First, protein-RNA interactions are covalently linked by in vivo UV crosslinking. Protein-RNA complexes are purified using oligo(dT), applying very stringent washes. Finally, the repertoire of RBPs differentially regulated by HIV-1 is determined by quantitative proteomics of uninfected versus infected cells.

As ~20% of the mRNA in HIV-1 infected cells is viral RNA, we expect that many of the upregulated RBPs will be interacting with this abundant transcript. On the other hand, HIV-1 is expected to shut of cellular functions that may impair viral replication.

Our study provide a comprehensive set of host proteins with differential RNA binding activity in mock and HIV-1 infected cells.

Poster – 08

Molecular dynamics simulations of hydrophobic gating in the TMEM175 channels
by Charlotte Lynch

Nanopores are nanoscale apertures in membrane proteins through which water and ions can pass. One such nanopore structure is TMEM175 [1], a tetramer containing a constriction bounded by three rings of hydrophobic amino acid residues (isoleucine and two leucines). These residues act as a hydrophobic gate [2] preventing water from passing through the pore without steric occlusion of the structure. By using molecular dynamics simulations we show that the pore lining wets or de-wets as a function of pore radius and the hydrophobicity of its lining.

Furthermore, since the water-water and water-lining interactions are of paramount importance to hydrophobic gating, we investigate the sensitivity of wetting/de-wetting across a range of different water models. In doing so, we offer insights into the molecular interactions taking place within the pore and the suitability of different water models for investigating hydrophobic gating.

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2. Trick, J.L., Chelvaniththilan, S., Klesse, G., Aryal, P., Wallace, E.J., Tucker, S.J. & Sansom, M.S.P. (2016) Functional annotation of ion channel structures by molecular simulation. Structure 24: 2207–2216.

Poster – 09

Towards unravelling tertiary structure of a mammalian long non-coding RNPs
by Jeongyoon Choi

Long non-coding (lnc) RNAs play crucial roles in many biological processes in the cell. Recent studies have suggested that many lncRNAs form ribonucleoprotein particles (RNPs), complex of RNAs and proteins, to function in the cell. One key example is Xist lncRNA, a master regulator of X chromosome inactivation in female mammals.

Recent genetic and proteomic screening studies have provided a list of Xist RNA binding proteins (Xist RBPs). Cells depleting key Xist RBPs show reduced activity in X chromosome silencing. Secondary structural analysis of partial Xist RNAs have been performed by several research groups. However, we still lack understanding of tertiary structure of native Xist RNP. Here, we will discuss our efforts and progress in unravelling tertiary structure of the Xist RNP.

Poster – 10

"Stick 'em with the pointy end": how bacteria evolved to stab
by William Smith

Resembling a spring-loaded poison needle, the T6SS (Type-VI Secretion System) enables Gram-negative bacteria to outcompete rivals by injecting them with toxins. Bacteria wield T6SS weaponry in different ways: whereas many species respond to threats by firing T6SS needles randomly in space, the opportunistic pathogen Pseudomonas aeruginosa shoots in retaliation, sensing incident T6SS attacks and returning fire on assailants. While the regulatory proteins controlling this response are known, we still understand little of when or why retaliation is preferable to random firing. Here, we use computational modelling to compete different T6SS fighting strategies in communities, characterizing the conditions necessary for each to evolve. Corroborated by experiments in vitro, our model predicts that retaliator success is contingent on the ability to launch multiple counter-attacks, allowing exploitation of the spatial information provided by attack sensing. Overall, our work sheds light on the strengths and weakness of different T6SS strategies, improving our understanding of this ubiquitous and potent bacterial weapon.

Poster – 11

Systematic Allelic Analysis Defines the Interplay of Key Pathways in X Chromosome Inactivation
by Guifeng Wei

Xist RNA, the master regulator of X chromosome inactivation, acts in cis to induce chromosome-wide silencing. Whilst recent studies have defined candidate silencing factors, their relative contribution to repressing different genes, and their relationship with one another is poorly understood. Here we describe a systematic analysis of Xist-mediated allelic silencing in ES cell-based models. Using a machine learning approach we identify distance to the Xist locus and prior gene expression levels as key determinants of silencing efficiency. We go on to show that Spen, recruited through the Xist A-repeat, plays a central role, being critical for silencing of all except a subset of weakly expressed genes. Polycomb, recruited through the Xist B/C-repeat, also plays a key role, favouring silencing of genes with pre-existing H3K27me3 chromatin. LBR and the Rbm15/m6A-methyltransferase complex make only a minor contribution to gene silencing. Together our results provide a comprehensive model for Xist-mediated chromosome silencing.

Poster – 12

Development, calibration and validation of a novel computational human ventricular cardiomyocyte model in healthy, diseased, and drug block conditions
by Jakub Tomek

Human-based modelling and simulation are becoming ubiquitous in biomedical science, due to their ability to augment experimental and clinical investigations. Cardiac electrophysiology is one of the most advanced areas, with cardiac modelling and simulation being considered for virtual testing of pharmacological therapies and medical devices.

In this study, we present the design, development, calibration and independent validation of a human-based ventricular model (ToR-ORd) for electrophysiology and excitation-contraction coupling simulations, from ionic currents to the electrocardiogram, able to reproduce a wide range of recordings in healthy and key disease conditions and under drug block.

Methods and Results: We first design the independent model development calibration-validation strategy based on an extensive list of criteria. Secondly, we evaluate the gold-standard O'Hara-Rudy model, and identify inconsistencies, primarily related to sodium and calcium dynamics. Thirdly, we perform an in-depth evaluation of biophysical properties of the L-type calcium current, leading to its reformulation and new theoretical insights, the hERG model replacement, and alterations in other currents. Fourthly, a multicriterial genetic algorithm optimisation is applied to calibrate the parameters in the new ToR-ORd model to fulfil imposed criteria. Finally, we conduct validation through comparison with independent experimental recordings, demonstrating that ToR-ORd-based simulations are capable of reproducing alterations caused by drugs and disease, transmural heterogeneities, as well as realistic activation and repolarisation patterns and electrocardiograms in biventricular simulations.

Conclusions: Based on independent development/calibration/validation, a new human ventricular ToR-ORd model is presented with its credibility supported by substantial multiscale simulations in healthy and disease conditions as well as drug response. Beyond the model itself, theoretical results on the properties of L-type calcium current and hERG are broadly relevant for computer models across cell types and species.

Poster – 13

Characterising early events following acute ablation of PRC1 in mouse embryonic stem cells
by Paula Dobrinic

Polycomb group proteins (PcGs) play an important role in regulation of transcriptional programs underlying normal mammalian development. PcGs function by forming a number of distinct multi-protein complexes which frequently co-occupy unmethylated CpG islands and reinforce each other's recruitment and activity, resulting in a formation of transcriptionally repressive chromatin domains. Recent findings from our group emphasize the role of one class of PcG complexes, Polycomb Repressive Complexes 1 (PRC1) in the formation of repressive Polycomb domains. PRC1 acts through monoubiquitylation of histone H2A on lysine 119 (H2AK119ub1) and formation of higher order chromatin structures, although exact mechanisms of transcriptional silencing still haven't been elucidated. Previous studies in mouse embryonic stem cells established that genes involved in differentiation and developmental processes are preferentially upregulated in the absence of the core components of PRC1. However, these findings were based on conventional genetic deletion approaches which preclude discovery of primary targets due to the time needed for complete degradation of both mRNA and protein, usually on a scale of days. We used an alternative perturbation system based on auxin-inducible degradation of the PRC1 catalytic subunit which enabled us to detect transcriptional changes happening on a scale of hours. The core PRC1 subunit depletion resulted in a very rapid loss of H2AK119ub1, and immediate and global gene upregulation. Detailed characterisation of direct targets of a major transcriptional regulator, such as PRC1, is required to understand its function and mechanisms behind establishment and maintenance of cellular identity, as well as the consequences of its malfunction in disease.

Poster – 14

Investigating the perturbation of neural crest cells in diabetic embryopathy
by Nikita Ved

Diabetes mellitus is a major health issue worldwide, with 425 million affected individuals. It is well understood that diabetes can result in a wide range of serious complications.

However, it is less well known that maternal diabetes during pregnancy increases the risk of having a child with a birth defect by over 4-fold, such that 10% of infants of diabetic mothers have a birth defect. This is termed diabetic embryopathy. Certain types of birth defects are more prevalent: congenital heart disease; craniofacial defects, including cleft lip/palate and micrognathia; and neural tube defects, including anencephaly, exencephaly and spina bifida. We have established a unique inducible and reversible mouse model of diabetic embryopathy that induces exactly the same range of embryonic defects. The specific types of heart and craniofacial defects observed strongly suggest a perturbation of neural crest cells (NCC). We have also shown that, if diabetes is reversed peri-implantation, we can rescue the neural crest phenotypes. We are delineating whether these perturbations are due to a dysfunction in NCC proliferation, migration or differentiation by using a combination of morphological examination with μMRI and histology, and an unbiased molecular investigation using transcriptomics.

Poster – 15

How bacteria coordinate outer membrane invagination with septation
by Joanna Szczepaniak

Bacterial cell division (septation) is driven by formation of the divisome, a multiprotein mid-cell assembly that synthesises peptidoglycan and coordinates invagination of both the inner and outer membranes of Gram-negative bacteria. How these events are synchronised and the molecular mechanisms involved are largely unknown. The present work focuses on the five-protein Tol-Pal assembly that is found in most species of Gram-negative bacteria, traverses the two membranes, is coupled to the proton motive force (PMF) and is recruited to the divisome late during septation. Since the 1960s Tol-Pal has been implicated in outer membrane stabilisation, but the assembly has also been implicated in other cell envelope functions such as peptidoglycan biogenesis and phospholipid transport. Here, using a combination of fluorescence live cell imaging, cell biological and structural approaches, we show that Tol-Pal is indeed required to stabilise the outer membrane during division but by a mechanism yet to be described in bacteria. The role we have uncovered for Tol-Pal explains the requirement for the PMF and how outer membrane invagination is coordinated with septation.

Poster – 16

Acetyl-CoA-Carboxylase 1 (ACC1) plays a critical role in glucagon and GLP1 secretion, and controls whole body glucose homeostasis.
by Anna Veprik

Background: One of the hallmarks of diabetes is dysregulated glucagon secretion from pancreatic alpha cells. Glucagon is cleaved from proglucagon, giving rise also to Glucagon-Like Peptide 1 (GLP1). GLP1 is secreted from gut enteroendocrine L-cells, potentiating insulin secretion, and GLP1 levels are reduced in type 2 diabetes. Despite the important role of these cell types in glycaemic control, the mechanisms regulating their secretory function are not well defined. We have previously identified that Acetyl-CoA-Carboxylase 1 (ACC1), the rate-limiting enzyme of de-novo lipogenesis, reduces beta cell growth and insulin secretion in a model of a specific deletion of ACC1 in pancreatic beta cells. The aim of the current project was to explore the role of ACC1 as a general modulator of secretory activity in pancreatic alpha and gut L-cell function and its effect on the organism metabolism.

Results: To investigate these mechanisms in vivo, we generated mice with ACC1 ablated in alpha and L cells using the glucagon driven Cre-Lox system (gluACC1-KO). Specific deletion was observed in the islets and in the gut. gluACC1-KO mice showed no changes in body weight, fasting glucose, random fed glucose and in food administration. However, they were glucose intolerant relative to littermate control mice, with lower fasting serum glucagon levels that were unresponsive to glucose administration. Correspondingly, the secretion of glucagon, but not insulin, from islets isolated from the gluACC1-KO mice was severely impaired in low glucose condition, relative to controls, and active GLP1 secretion from primary gut crypts was reduced in the gluACC1-KO mice. Consistent with this, pharmacological inhibition of ACC impaired glucagon secretion from transformed alpha cells, primary mouse and human islets, as well as impairing active GLP1 secretion from transformed enteroendocrine cells and primary gut crypts.

To elucidate the secretion defect in gluACC1-KO islets the secretion machinery was examined. The altered secretion was unaffected by modulation of the KATP channel activity while membrane depolarisation by KCl induced glucagon secretion in the gluACC1-KO islets. Although glucagon secretion was

increased, following membrane depolarisation, it failed to reach the levels of secretion in Ctrl islets which might be explained by reduction in the total glucagon content per islet in the gluACC1-KO mice.

Summary: Our data reveals a critical role for ACC1 in controlling alpha and L-cell function and whole body glucose homeostasis.

Poster – 17

STUDYING GENE REPRESSION USING HIGH-THROUGHPUT 3D MICROSCOPY
by Aleksander Szczurek

Most of the eukaryotic genes are transcribed in so called transcription bursts, i.e. short periods of time when gene promoter becomes permissive allowing the production of one to dozens of transcript copies at once. Bursts are typically followed by relatively long periods of transcriptional inactivity. This feature of transcription introduces additional variability in the population of genetically identical cells (transcription noise) resulting in distribution of transcription levels more dispersed than Poisson distribution typical for constitutively expressed genes.

Polycomb group proteins have a major role in mammalian embryonic development and cell differentiation through repression of their target genes. Whether their repressive function stems from alteration (decrease) of the transcription burst size or frequency still remains poorly understood. Moreover, whether Polycomb-repressed genes are uniformly lowly expressed in the entire cell population remains unknown and has not been studied likely due to technical caveats. Namely, to accurately assess transcription level distribution of repressed genes hundreds to thousands of single cells have to be studied.

Therefore, we employed high-throughput 3D widefield microscopy in combination with single molecule fluorescence in situ hybridisation (smRNA-FISH) of repressed genes in mouse embryonic stem cells. This approach allowed us to count individual molecules of transcripts and capture relatively rare cells (1:100 cells) to obtain accurate information about single-cell variability in a population. We demonstrate that the repressed genes have higher transcription noise than actively transcribed genes. Furthermore, fitting two-state model of transcriptional regulation to single-cell transcription level distributions provided new insights into mechanistic aspects of gene repression mediated by Polycomb group proteins.

Poster – 18

Differences in extracellular matrix deposition during heart regeneration and scarring in Astyanax mexicanus
by Zhilian Hu

The cardiac regenerative capacity of Astyanax mexicanus populations is completely distinct in response to the injury. Surface fish can regenerate the ablated heart tissue, similar to zebrafish, while Pachón cannot and instead forms a permanent scar with excess extracellular matrix (ECM), similar to human.

ECM is a dynamic three-dimension network of extracellular macromolecules including collagen and non-collagenous components. Its content, relative proportion, and spatial arrangement play a pivotal role during tissue regeneration. Any changes in ECM deposition can have a big impact on the ability to regenerate. Astyanax mexicanus allows us to identify the differences in ECM deposition and removal between a degradable scar in a regenerative setting and a permanent scar in a non-regenerative setting.

Our research indicates ECM components and their regulating Matrix metalloproteinases (MMPs) are dynamically and differentially expressed during heart regeneration between surface fish and Pachón. Next, we will link the key scarring differences directly to both the genome and the ability for heart regeneration using new and prior Quantitative Trait Loci analyses. This will allow to find the most fundamental molecular mechanisms directing the wound healing process towards regeneration versus scarring.

Poster – 19

Gene regulation by PCF11-dependent alternative polyadenylation and transcription termination
by Kinga Kamieniarz-Gdula

The pervasive nature of RNA polymerase II (Pol II) transcription requires efficient termination. A key player in this process is the cleavage and polyadenylation (CPA) factor PCF11, which directly binds to the Pol II C-terminal domain and dismantles elongating Pol II from DNA in vitro. We demonstrate that PCF11-mediated termination is essential for vertebrate development. A range of genomic analyses, including: mNET-seq, 3’ mRNA-seq, chromatin RNA-seq and ChIP-seq, reveals that PCF11 enhances transcription termination and stimulates early alternative polyadenylation

genome-wide. PCF11 binds preferentially between closely spaced genes, where it prevents transcriptional interference and downstream gene silencing. Notably, we show that PCF11 is sub-stoichiometric to the CPA complex and acts as a regulatory factor. Low levels of PCF11 are maintained by an auto-regulatory mechanism involving premature termination of its own transcript, and are important for normal development. Both in human cell culture and during zebrafish development, PCF11 selectively attenuates also the expression of other genes by premature CPA and termination. Strikingly, this PCF11-dependent attenuation targets preferentially transcriptional regulators, with the same genes prematurely terminated by PCF11 in human and zebrafish. The phenomenon of negative gene regulation by premature termination is well described in S. cerevisiae, where it plays a physiological role in response to changing growth conditions. We propose that premature termination is a fundamental gene regulatory mechanism operating also in vertebrates.

Poster – 20

Molecular basis for Aurora B transport from chromatin to the central spindle in anaphase
by Michela Serena

The chromosome passenger complex (CPC) is composed of different proteins: Survivin, Borealin, Incenp and Aurora B kinase. It plays important roles during mitosis, which are related to its localisation, which is very dynamic during the cell cycle. In particular, during metaphase, the CPC localises at centromeres, where Aurora B is involved in controlling the spindle assembly checkpoint. Afterwards, during anaphase, the CPC is relocated to the central spindle, upon binding to the MKlp2 kinesin, where Aurora B is involved in controlling cytokinesis. However, the details of the mechanism underlying this process, which has to be tightly regulated in time and space, are still poorly understood. In this work we untangle how the CPC specificity for the centromeric histone H3-Thr3P is achieved in metaphase and how the CPC localisation at the central spindle is then promoted in anaphase. In particular, we characterise the crystal structure of the CPC bound to a 12 amino acids long H3-Thr3P peptide, and we also demonstrate that the Incenp patch of positively charged amino acids from 63 to 70 includes a DNA binding motif which strengthens CPC centromere localisation. Our data point out that this region is also necessary for MKlp2 binding and CPC relocation to the central spindle in anaphase, thus ensuring a mutually exclusive spatio-temporal regulation of CPC localisation throughout mitosis. In addition, we also define the MKlp2 region necessary for CPC binding, showing that after their association, MKlp2 ATPase activity is increased. Finally, NMR allowed us to characterise the molecular details of CPC/MKlp2 interaction.

Poster – 21

Translocation Mechanisms of Protein-Antibiotics
by Ruth Cohen Khait

Bacteriocins are peptides or proteins with antimicrobial activity that contribute to the stability and dynamics of microbial communities. In an era of multidrug resistant bacteria, bacteriocins may have utility as natural antibiotic alternatives. However, little is known about the molecular mechanisms of their cellular import. Here, we follow the dynamic translocation process of two colicins, ColB, a pore-forming toxin, and ColD, a nuclease, which parasitize the same Escherichia coli receptor (FepA) yet deliver toxic domains to different cellular compartments. Our multidisciplinary study combines photoactivatable crosslinking LC-MS/MS, structural approaches (cryo-electron microscopy and X-ray diffraction) and live cell fluorescence microscopy to understand how these potent protein antibiotics cross one or two membranes of the bacterium and the energetics of these processes.

Poster – 22

Single-molecule assays explore the mechanism of chromosome organization by the E.coli SMC complex, MukBEF
by Man Zhou

Although Structural Maintenance of Chromosomes (SMC) protein complexes play crucial roles in chromosome organization in all domains of life, the molecular mechanism of their action remains unclear. Here, we exploit two complementary single-molecule assays to probe the function of the E. coli SMC complex, MukBEF. In assay 1, MukB was immobilized by using a His6-tagged variant of MukB and biotinylated anti-His6-antibody on the polyethylene glycol (PEG) modified surface, and interaction of either linear or circular DNA

with the immobilized MukB(EF) was analyzed. In assay 2, 48 kbp λDNA was doubly tethered to the PEG modified surface by introducing biotin at its two termini, and the interactions of MukB(EF) with this DNA were studied. In both assays, relatively weak interactions of MukB alone with DNA could be observed. MukB head-engagement prior to exposure to DNA lacking free ends impaired the interaction. With a high density of immobilized MukB, multiply tethered circular DNA were seen. On laser-induced DNA breakage, sequential sliding of the linear DNA through the tethered MukB complexes was observed. Once MukB had interacted with DNA, addition of MukEF-ATP lead to the stabilization of the DNA-bound complexes against high salt, with evidence of local DNA compaction by MukB(EF). We also observed some trapped DNA loops, but have not obtained evidence for active DNA transport or loop extrusion.

Poster – 23

Structural basis of malodour precursor transport by bacterial POT transporters
by Gurdeep Minhas

Proton-coupled transporters (POT/PTR family) drive the uptake of di- and tri- peptides across cellular membranes. POT transporters are also capable of transporting small non-physiological peptidomimetic substrates such as antibiotics (β-lactams) and antiviral drugs (Valacyclovir).

Bacterial POT transporters, however, can also function as xenobiotic transporters. An example xenobiotic substrate, shown here, is the malodour precursor Cys-Gly-3M3SH (CG3M3SH), which is metabolised by the commensal bacteria, Staphylococcus hominis, to produce a pungent thioalcohol by-product familiarly known to us as body odour. Here, we present the high-resolution crystal structure of the S. hominis POT transporter (PepTHom) in complex with the malodour precursor CG3M3SH. Our study identifies a novel hydrophobic pocket responsible for CG3M3SH recognition and reveals why this particular Staphylococci bacterium is solely responsible for the production of human body odour.

Poster – 24

PRC1 E3 ligase activity is essential for Polycomb-mediated gene repression
by Neil Blackledge

Polycomb group proteins are chromatin-based transcriptional repressors that are essential for normal gene regulation during development, while mutations affecting Polycomb proteins are a hallmark of many cancers. In mammals, Polycomb proteins generally fall into one of two central complexes: Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). Importantly, both of these complexes have chromatin modifying activities: PRC1 has E3 ligase activity that mono-ubiquitylates histone H2A at lysine 119 (to form H2AK119ub1), while PRC2 methylates histone H3 at lysine 27 (to form H3K27me1/2/3). However, the precise contribution of these histone modifying activities to the Polycomb system remains poorly defined. This situation is especially complicated in the case of PRC1, for which H2AK119ub1-dependent and –independent processes have been proposed.

To establish a better mechanistic understanding of the Polycomb system in mouse embryonic stem cells, here we make a series of mutations, both constitutive and conditional, that specifically ablate the E3 ligase activity of PRC1 without affecting the integrity of the PRC1 complex. Using quantitative genomic approaches, we go on to systematically dissect the effect of these mutations on various aspects of the Polycomb repressive system. This reveals that PRC1 E3 ligase activity is essential for communication between PRC1 and PRC2, ultimately driving localisation of both PRC1 and PRC2 to repressed gene promoters. Most importantly, we show that in mouse embryonic stem cells, PRC1 E3 ligase activity is absolutely essential for Polycomb-mediated gene repression. These discoveries pave the way for a detailed understanding of the molecular mechanisms that underpin Polycomb target site selection and gene repression.

Poster – 25

Imaging organisation in the Escherichia coli outer membrane
by Sandip Kumar

Antibiotic resistance in bacteria is on the rise for all classes of antibiotic. In the case of pathogenic Gram-negative bacteria, many are intrinsically resistant to some classes of

antibiotics due to the outer-membrane (OM) acting as a diffusion barrier for drug molecules. Hence understanding how the OM is built and maintained offers new routes for combatting antimicrobial resistance. The OM is asymmetric, with an outer leaflet of lipopolysaccharides (LPS) and an inner leaflet of phospholipids. The predominant proteins within this asymmetric bilayer are β-barrel outer membrane proteins (OMPs). While a great deal is known about the biogenesis of OMPs and LPS and how they are deposited on the surface of Gram-negative bacteria, surprisingly little is known about how these molecules are organised relative to each other in a live bacterium. Using OMP-specific fluorescent probes, we recently discovered that OMPs are organised into large, supramolecular assemblies (OMP islands) the formation of which underpins the turnover of OMPs in bacteria. In the work to be presented, we combine diffraction limited fluorescence and super-resolution microscopy methods with specific labelling of OMPs and LPS to investigate their relative organisation in live bacteria and how these change during cell division and in response to environmental stress factors.

Poster – 26

FBXL19/CDK-Mediator prime developmental genes by promoting their interactions with regulatory sites
by Angelika Feldmann

The CxxC-domain protein FBXL19 plays a role in targeting CDK-Mediator to the promoters of silent developmental genes in mESCs and this is required for appropriate activation during differentiation. Based on these observations we proposed that FBXL19 primes gene promoters for activation through recruitment of CDK-Mediator. However, the mechanisms by which FBXL19 and CDK-Mediator prime genes for expression remain unknown. Mediator has previously been proposed to support long-range interactions between distal gene regulatory elements and promoters in mESCs. Therefore we reasoned that FBXL19 may elicit its transcriptional priming effect through shaping interactions between gene promoters and distal regulatory elements via CDK-Mediator. To test this hypothesis we first devise a stringent set of FBXL19 responsive genes and identify their interactions globally. We show that these genes interact with CDK8 enriched sites in an FBXL19 and intact CDK-Mediator dependent manner. Despite gaining activity and being required for gene induction, these sites do not interact with their cognate gene promoters in retinoic acid differentiated cells. Our findings suggest that FBXL19/CDK-Mediator prime developmental genes by regulating promoter interactions that are required in the transition between pluripotent and a more committed states.

Poster – 27

Structural characterisation of the essential fungal proton pump Pma1
by Sabine Heit

Invasive fungal infections are a major cause of death among immunocompromised patients worldwide. A potent target for the treatment of fungal infections is Pma1, a P-Type ATPase that pumps protons across the plasma membrane of fungi to create an electrochemical gradient essential for the secondary transport of nutrients. The aim of the current research is to obtain structural information of Pma1 at atomic resolution to gain insights into its structural and functional organisation and to enable the identification of a specific inhibitor.

Poster – 28

The topology of DNA entrapment by cohesin rings.
by Christophe Chapard

Cohesin entraps sister DNAs within tripartite rings created by pairwise interactions between Smc1, Smc3, and Scc1. Because the ATPase heads of Smc1 and Smc3 can interact with each other, cohesin rings in fact have the potential to form a variety of sub-compartments. Using in vivo cysteine crosslinking, we show that when Smc1 and Smc3 ATPases are engaged in the presence of ATP (E heads) cohesin rings generate a “SMC (S) compartment” between hinge and E heads and a “kleisin (K) compartment” between E heads and their associated kleisin subunit. Upon ATP hydrolysis, cohesin’s heads associate with each other in a very different mode, in which their signature motifs and their coiled coils are closely juxtaposed (J heads), creating alternative S and K compartments. We show that all four sub-compartments exist in vivo, that acetylation of Smc3 during S phase is accompanied by an increase in the ratio of J to E heads, and that sister DNAs are entrapped in J-K compartments.

Poster – 29

Exploring magnetically aligned bilayers as a novel tool for membrane protein crystallisation
by Nada Mohamad

Abstract TBA

Poster – 30

The MukBEF complex: single-molecule reconstitution of its transport of DNA and exploration of the role of conformational dynamics
by Gemma Fisher

SMC class proteins are increasingly being recognised as a major determinant of chromosome organisation. Like all SMC proteins, the E. coli SMC-like MukB complex displays a distinct architecture comprising a "hinge" dimerisation module and an ATP-hydrolysing "head" domain, connected by ~50 nm long antiparallel coiled coils "arms". MukB monomers associate to form homodimers, and multimers thereof, which adopt a range of conformations, as observed directly in AFM and EM studies. Secondary dimerisation occurs at the bipartite head domain upon ATP binding, which is reinforced by the binding of MukF and MukE, resulting in a proteinaceous ring. Models for SMC function often invoke the idea that this ring is topologically engaged with the chromosome. More recently, studies have also eluded to the idea that SMC proteins actively extrude loops of DNA. The molecular basis of this activity, and applicability to all SMC systems, is an area of active debate. In particular, the nature of the presumed multiple DNA contacts required for such activity and how this is modulated by ATP turnover and the regulatory kleisin and kite/hawk binding partners. In this work we describe development of single-molecule fluorescence-based assays to monitor putative DNA transport by the MukBEF complex in real-time. Additionally, we use single-molecule FRET analysis of full-length MukB, specifically labelled at the heads, coiled coils or hinges, to measure structural transitions at each of these domains relating to ATP turnover and/or the binding of the MukE and MukF complexes.

Poster – 31

Anthony Nash
Title/Abstract TBA

Poster – 32

Super-Resolution Microscopy on Centrioles and Centrosomes
by Alan Wainman

Super-resolution techniques have allowed us to examine cellular structures in increasing detail, but a full understanding of biological processes requires a dynamic dimension. Here, we describe how existing super-resolution imaging and analysis approaches can be combined in an innovative way to produce a more complete picture of a dynamic biological process, using the Drosophila centriole as an example.

Centrioles are organelles responsible for the organisation of the mitotic spindle. We have previously determined the precise localisation of its components, but their dynamic incorporation over time is yet to be explored. Crucially for our analysis, we combined: the gentle treatment of fluorescence samples by the super-resolution airyscan system, which allowed for extensive time-lapse super resolution imaging; the large number of synchronous centrioles in the Drosophila embryo; subsequent computational analysis of these large number of high resolution imaged centrioles. Our work provides a complete picture in all four dimensions of a dynamic cellular organelle, an approach that can be replicated in other systems.

Poster – 33

Structure determination and investigation of molecular assembly of C4bp: a negative complement regulator protein
by Nandita Bodra

The complement system is a part of the immune system. It enhances the ability of antibodies and phagocytic cells to clear microbes and damaged cells and promotes inflammation and attacks the pathogen's cell membrane. It consists of sequentially interacting proteins that provide a rapid and powerful host defense. About 60 proteins comprise three activation pathways namely classical, alternative, and lectin pathway and a terminal cytolytic pathway to form complement cascade. These pathways need to be tightly controlled to avoid damage to "self" cells and therefore complement system regulators are required. The C4bp is a negative regulator of classical and lectin pathway. C4bp has a complex structure, mainly comprises of alpha chains and a single copy of a beta chain. It also

possesses binding site for vitamin K dependent protein S. Here, my project aims to structurally characterize C4bp protein and its binding partner. To achieve this goal, I use insect cell expression system to produce the protein and X-ray crystallography to determine the structure. The outcome of this work will give insights on the molecular details of C4bp which will be helpful to design novel complement targeted therapeutic agents.

Poster – 34

CytoCensus: Live imaging and analysis in the developing brain
by Martin Hailstone

A major challenge in cell and developmental biology has been the automated identification and quantitation of cells in complex multilayered tissue landscapes. We have developed CytoCensus: an implementation of supervised machine learning that extends convenient 2D “point-and-click” user training to 3D identification of cells in challenging datasets with ill-defined cell boundaries. We used CytoCensus to count stem cells and their progeny, and quantify their individual cell divisions from time-lapse movies of explanted whole Drosophila larval brains. We illustrate the general utility of CytoCensus, analysing the organisation of multiple cell classes in zebrafish retinal organoids and quantifying the distribution of cell types in mouse embryos. CytoCensus opens the possibility of automated annotation with minimal training effort, at the single cell level from large datasets of complex tissue.

Poster – 35

You shall not pass - the structural basis for transmission blocking vaccines against malaria
by Frank Lennartz

The development of an effective malaria vaccine remains a major priority in the fight against global infectious disease. An approach with great potential is a transmission-blocking vaccine which induces antibodies that block the transmission of the parasite from infected humans to its mosquito vector, thereby stopping the cycle that is responsible for the spread of the disease. One of the most promising targets for such a vaccine is the malaria gamete surface protein Pfs48/45, which is essential for the parasites’ sexual development in the mosquito.

Here, we characterize the potential of this protein as vaccine target by raising a panel of monoclonal antibodies against Pfs48/45. We map the binding regions of these antibodies on Pfs48/45 and correlate the location of their epitopes with their transmission-blocking activity. We further present the structures of the C-terminal domain of Pfs48/45 bound to the two most potent transmission-blocking antibodies, providing molecular information about crucial target sites for vaccine development. Finally, we use this structural information to generate immunogens that, when coupled to virus-like particles for immunization, specifically display these key inhibitory epitopes. Taken together, these results represent the first step towards the rational, structure-guided design of a transmission blocking malaria vaccine.

Poster – 36

Developing microscope software with Python
by David Miguel Susano Pinto

We have created Microscope and Cockpit, two Python packages to dramatically reduce effort required for bespoke microscope development. Scientists that would rather be building microscopes are instead spending their time in low-level software development and GUI toolkits to control the microscopes. This software ends up being tied to the specifics of the devices, impossible to maintain, or incur ongoing licensing costs. Effort is wasted as scientists in different labs, and even in the same lab, keep independently implementing solutions to the same problems.

Microscope is a Python package that provides remote control of microscope devices, abstract base classes of different type of devices, and concrete classes for the devices we had access to. Remote control enables us to control arbitrarily complex microscopes by distributing devices across multiple machines, while the common interface enables us to swap devices from different vendors without changes to the underlying code.

On top of Microscope we have created Cockpit, a graphical interface for microscope control and running both simple and complex experiments. The Cockpit interface adjusts to the available devices, providing device independent access to a wide range of hardware. Experiments are written in Python and provide generic experiment types such as time lapse or Z-stacks, as well as much more

complex experiments, independent of the actual device details.

We have successfully deployed Cockpit on a number of bespoke microscope systems, and used Microscope in student projects and for the development of microscope tools.

Poster – 37

TransLEISHion: Leishmania Transportome dissection using CRISPR-Cas9
by Andreia Albuquerque Wendt

Leishmaniases are neglected tropical diseases, which cause chronic disability and poverty in 98 countries. No viable vaccines currently exist and there is an urgency to find better drugs. Membrane transporters are likely to be important for parasite viability as they mediate the import and export of nutrients, waste products and facilitate environmental sensing. There are ~250 transporters predicted in the Leishmania mexicana genome. I aim to generate a library of null mutants using CRISPR-Cas9 to assess which genes are required for survival in macrophages. To date I have targeted 46 genes and achieved 39 viable null mutants. Two of these presented a strong defect in growth ability in the promastigote stage. I have also tagged these proteins at both termini with mNeonGreen, to determine the subcellular localisations. As a result, 42 N-terminally tagged and 44 C-terminally tagged mutants were isolated and imaged in the vector stage of this parasite (promastigote) and mammalian stage (amastigote). In the promastigote stage, several of these proteins displayed clear localisation to mitochondria (3), pellicular membrane (2), lysosome (3), cell body (7), whilst others exhibited low or ambiguous mNeonGreen signal, perhaps consistent with the fact that this group of proteins showed, in previous studies, to be upregulated in the amastigote stage. Alternatively, it is possible that due to the multimembrane nature of these proteins, the appropriate trafficking of the tagged proteins is disrupted, resulting in its degradation in different sites of the lysosomal compartment. Currently, I am completing this dataset with the corresponding localization of these proteins in amastigotes. Next, I will determine the growth curves of the null mutants, as well as their survival rate in bone marrow derived macrophages. At the end of this project, I expect to produce a list of attractive biological targets for drug development as well as the elucidation of their role in drug resistance.

Poster – 38

RNA-Mango: A fluorogenic aptamer for the cellular imaging of RNA
by Adam Cawte

In recent years, there has been an explosion of SELEX- evolved fluorescent RNA aptamers, such as Spinach, Broccoli, Corn and Mango. Fluorogenic RNA aptamers have sparked a lot of interest and hold great potential to enable background free visualisation of RNA molecules in a cellular environment. However, their application has been limited given their potential, mainly due to poor folding stability and fluorescent stability. Therefore, evolving new RNA aptamers with improved physico-chemical properties should better their use in cellular imaging.

Here I will present my previous work on imaging short non-coding RNAs, mRNAs and lncRNAs with photo-physically improved RNA-Mango aptamers. I will also highlight the focus of my proposed research toward developing the methodology to image and purify lncRNAs involved in the modulation of genome architecture.

Poster – 39

Designing Accelerated Protein Superglue for Molecular and Cellular Nanoassembly
by Dr Anthony Keeble

Assembly of large precise nanostructures using non-covalent interactions is limited by the kinetic instability of the complexes. With the aim of producing nanostructures that assemble with infinite affinity, we previously engineered adhesion proteins from the bacterium Streptococcus pyogenes that spontaneously form isopeptide bonds, to produce a genetically-encoded and highly specific protein coupling platform. The resulting SpyCatcher protein fused to one protein irreversibly forms a protein-peptide bond with the SpyTag peptide fused to another protein. However, one limitation of the technology has been the slow reaction at low concentrations. We have now used directed evolution by phage display and computer-guided rational design to create SpyCatcher-SpyTag pairs that react with rates close to the diffusion limit, orders of magnitude faster than before. Rapid reaction occurs even at low nanomolar concentrations, with the mechanistic basis identified by a range of biophysical analyses. Reaction occurs through a range of conditions (pH, temperatures and buffers) including relatively harsh conditions (4

M urea) in addition to milder, cellular mimicking conditions. We also generated a reversible SpyCatcher enabling affinity purification of SpyTag-ligands. Various applications have been enabled by SpyCatcher-SpyTag, including making enzymes resilient to boiling, multi-enzyme scaffolding, bacterial and mammalian cell labeling, rapid functionalization of antibodies, and nanoassembly for malaria vaccine development.

Poster –40

Unmasking the heterogeneity of human DNA replication
by Jamie Carrington

Eukaryotes regulate DNA replication by controlling the activity of a large number of replication origins; protein-bound genomic loci from which DNA replication has the potential to initiate. This control is executed in terms of the genomic locations, the frequencies, and the timings of origin activations in a given S phase. It is estimated in human cells that ~180,000 origins are variably activated to duplicate ~6 billion basepairs every cell cycle. Exactly how cells regulate such an essential and complicated process is not yet clear. In budding yeast the location of replication origins are defined by a consensus sequence, aiding the characterisation of both their frequency and timing of activation as well. However, in human cells there is no replication origin consensus sequence, and the many attempts that have been made to map their genomic locations has revealed that their positioning is highly stochastic. Origin-poor and -rich, and early- or late-replicating regions of the genome have been described, but because cell population studies mask the natural heterogeneity of human DNA replication it is difficult to understand how the regulation of individual origins translates into this observed regional variability. Efforts have been made to observe the activity of individual origins by combing long molecules of DNA and immuno-labelling nucleotide analogues incorporated by DNA polymerases, from which origin position can be derived, however, this assay is difficult to scale and does not inform on genomic location. Recently, the Nieduszynski lab developed a method to detect nucleotide analogues by nanopore sequencing of long DNA molecules (D-NAscent). There are three advantages of detecting nucleotide analogues by nanopore sequencing; active DNA replication is detected within the context of the genome sequence, very long single molecules are analysed, and it is scalable to the whole genome. Using D-NAscent, origin activity can be located in many individual molecules of DNA that cover the entire genome, which combines the advantages of the scale of cell population studies with the precision of individually combed DNA molecules. I will present preliminary data which indicates that D-NAscent can detect tracts of BrdU incorporation in human cell genomic DNA, as well as how D-NAscent will be used to find origins activated in early-S phase and a limiting concentration of BrdU will be used to derive replication fork direction. D-NAscent has the potential to unveil a description of stochastic origin location, and frequency and timing of activation in human cells with unprecedented scale and depth.

Poster – 41

Towards a mechanistic understanding of T cell adaptation to antigen in the presence of accessory receptors
by Robert Smith

T cells are activated to respond when their surface T cell antigen receptors (TCRs) recognise foreign antigens in the form of short peptides bound to major histocompatibility complexes (pMHC). The binding of pMHC to the TCR can trigger a signalling pathway that can induce T cells to secrete cytokines such as interferon- γ (IFN-γ), and downregulation of the TCR from the surface of T cells. After 4 – 8 hours these processes stop, a phenomenon termed adaptation. In addition to the TCR, it is now appreciated that other, accessory, surface receptors can also control cytokine production. Our data shows that the presence of co-stimulatory ligand CD70 (that binds CD27 on a T cell) leads to increased IFN-γ levels being produced prior to adaptation, whilst the presence of 4-1BBL (that binds 4-1BB on a T cell) abrogates the adaptation of cytokine secretion by CD8+ effector T cells. Thus it is unclear how two members of the TNF superfamily mechanistically regulate adaptation to produce different phenotypes. Here, we use phenotypic pathway models to unravel the mechanism underlying these different phenotypes on T cell responses. We first obtain a model that is able to describe adaptation in the absence of accessory receptor ligands with a focus on underlying parameter identifiability in the face of human donor variability. Next, we attempt to identify the integration point of CD27 and 4-1BB into the pathway model that allows them to reproduce their observed phenotypes. Finally, we attempt to understand the different

possible ways in which accessory receptors can influence adaptation at specific points in the network. These results provide a better understanding of the cross-talk different receptor pathways have on T cell behaviour, with the potential for the rational design of therapies targeting these accessory receptors.

Poster – 42

Delayed adaptation to oxidative stress causes a mutagenesis pulse in E. coli
by Valentine Lagage

In an oxygen-rich environment, cells are constantly exposed to Reactive Oxygen Species (ROS). Bacteria encounter sudden bursts of ROS that are generated by immune cells and antibiotic treatments. Hydrogen peroxide (H2O2) can damage all biomolecules including DNA through the formation of hydroxyl radicals (HO.) by the Fenton reaction. Hence, bacteria employ multiple parallel mechanisms to prevent and repair oxidative damages. In E. coli, the transcriptional regulator OxyR induces the expression of many proteins including ROS scavenging enzymes (such as ahpC peroxidase) to eliminate intracellular H2O2. Degradation of oxidized nucleotides (8-oxoG) by MutT dephosphatase and the action of several DNA repair pathways are important for the maintenance of DNA integrity. Because oxidative DNA damage is highly mutagenic and cytotoxic, rapid activation of the oxidative stress response must be critical when bacteria are suddenly exposed to ROS. Here, we employ our recently developed microscopy method to monitor the dynamics of mutagenesis and cell survival in response to H2O2. Using microfluidics, we were able to link mutagenesis with the gene regulatory dynamics of the oxidative stress response in single cells

Poster – 43

Optimisation of a high-content phenotypic screening method for studying macrophage phagocytosis.
by Annemieke Kok

Background: There is an unmet clinical need for new drugs which can reduce inflammation and induce healthy tissue repair mechanisms in chronic inflammatory diseases such as atherosclerosis, rheumatoid arthritis and diabetes. Macrophages play a key role in the initiation and progression of chronic inflammation, but also in tissue homeostasis, so modulation of their activity in vivo is an interesting drug target.

Methods: In order to identify small molecules which either inhibit or enhance macrophage phagocytosis, we have optimised a high-content, fluorescent, live cell imaging (IncuCyte ZOOM®) assay of macrophage phagocytosis, using phagocytic 'meals' labelled with pH-sensitive pHrodo dyes.

Results: Using unstimulated (M0) mouse BMDMs and known inhibitors of phagocytosis, phagolysosome acidification and cytoskeletal rearrangement, we have established dose-response curves, IC50 values, and Z'-scores for our assay using pHrodo-labelled, unopsonised heat-killed E.coli. Recently we have assessed the same parameters using different phagocytic 'meals', and macrophages polarised towards the M1 or M2 phenotype.

Conclusions: Further work will establish the utility of this phenotypic screening assay in identifying novel inhibitors and enhancers of macrophage phagocytosis by screening a library of FDA-approved anti-inflammatory medicines.

Poster – 44

SmcHD1 antagonises TAD formation and compartmentalisation on the inactive X chromosome independent of transcription
by Michal Gdula

The inactive X chromosome (Xi) in female mammals adopts an atypical higher-order chromatin structure, manifested as a global loss of local topologically associated domains (TADs), A/B compartments and formation of two mega-domains. Here we demonstrate that the non-canonical SMC family protein, SmcHD1, which is important for gene silencing on Xi, contributes to this unique chromosome architecture. Specifically, allelic mapping of the transcriptome and epigenome in SmcHD1 mutant cells reveals the appearance of sub-megabase domains defined by gene activation, CpG hypermethylation and depletion of Polycomb-mediated H3K27me3. These domains, which correlate with sites of SmcHD1 enrichment on Xi in wild-type cells, additionally adopt features of active X chromosome higher-order chromosome architecture, including A/B compartments and partial restoration of TAD boundaries. Xi chromosome architecture changes also occurred following SmcHD1 knockout in a somatic cell model, but in this case, independent of Xi gene derepression. We conclude that SmcHD1 is a key factor in

defining the unique chromosome architecture of Xi.

Poster – 45

R47H mutation of TREM2 selectively affects inflammatory activation in iPSC-macrophages
by Hazel Hall-Roberts

Triggering Receptor Expressed on Myeloid cells 2 (TREM2) is a microglia-specific gene, with mutations linked to Alzheimer's disease (AD), including R47H which increases the risk of AD by 2 to 4.5-fold (1, 2). TREM2 is a surface receptor that recognises anionic lipid "eat me" signals on apoptotic cells, and activates Syk-dependent intracellular signalling. Syk signalling triggers phagocytosis, and affects the activation state and survival of microglia. The R47H mutation has been reported to disrupt ligand binding and is therefore hypothesised to cause a reduction in TREM2 function (3). As a validated model of human microglia (4), we generated human induced pluripotent stem cell (iPSC)- derived macrophages. R47H TREM2 mutant iPSC-macrophages were studied alongside TREM2 knockout and wildtype iPSC-macrophages. Mutations had been introduced to healthy isogenic iPSCs by CRISPR gene-editing, allowing control of the genetic background. We investigated phenotypic responses towards neuronal death, using apoptotic SH-SY5Y cells and rat cortex synaptosomes as stimuli. Phenotypic responses included efferocytosis (phagocytosis) of the stimuli, downstream protein phosphorylation, and inflammatory cytokine secretion. Knockout of TREM2 impaired efferocytosis and downstream Syk signalling, and additionally had a detrimental effect on macrophage survival. In contrast, the R47H mutation had no significant impairment of efferocytosis or survival, yet impacts upon inflammatory cytokine production in the presence of neuronal debris. We propose that the R47H mutant affects specific functions of TREM2, but retains sufficient functionality to allow normal phagocytosis and pro-survival signalling to continue.

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Poster – 46

Factors affecting template switch recombination associated with restarted DNA replication
by Judith Oehler

The timely completion of DNA replication in each cell cycle is essential to avoid potentially catastrophic chromosome missegregation during mitosis. Replication forks (RFs) encounter numerous obstacles during their task of genome duplication, which can cause them to stall or even render them unable to continue DNA replication (referred to as fork collapse). If a RF collapses, DNA replication can be completed by the opposing fork, owing to the bidirectional nature of replication. Alternatively, collapsed forks can be restarted through the intervention of homologous recombination (HR). Recombination-dependent replication (RDR) is associated with high rates of template switching (TS) and, therefore, while it can aid the timely completion of genome duplication, it risks generating genome rearrangements.

Our lab has been studying HR dependent RF restart in the fission yeast Schizosaccharomyces pombe using an experimental system in which RF collapse is caused by a site-specific, polar replication fork barrier (RFB) called RTS1. Fork collapse at RTS1, which occurs without breakage of the fork, leads to RDR and associated TS that can be analysed using a direct repeat reporter positioned at or downstream of the barrier. Using this system, we found that RFB induced TS can be measured as far away as 75 kb downstream of the barrier, which contrasts with break induced replication (BIR) in S. cerevisiae where TS appears to be limited to a region of only 10 kb from the DNA break. We have also investigated the impact of different recombination factors on the frequency of TS. We have found that several DNA helicases (Pfh1, Srs2, Rqh1 and Fbh1) suppress RDR dependent TS, while the FANCM-like helicase Fml1 and Mus81 nuclease have no effect. Additionally, we identified that Pfh1 assists in fork restart – possibly in a manner akin to its counterpart in S. cerevisiae Pif1, which promotes D-loop migration during BIR. Finally, we found that TS is stimulated when restarted replication encounters transcription head-on.

Poster – 47

An anti-arrhythmic potential of of infarct border zone sympathetic innervation via alternans attenuation
by Jakub Tomek

Ventricular fibrillation, one of the leading causes of mortality worldwide, is typically present in patients with a history of myocardial infarction. Myocardial infarction triggers a broad range of remodeling, particularly in the infarct border zone, such as cellular decoupling, channel remodeling, and changed density of sympathetic nerve fibers. Traditionally, sympathetic hyperinnervation of the infarct border zone has been considered to be proarrhythmic, but recent human studies suggest that lack of innervation is proarrhythmic instead. There is thus a major clash of evidence which warrants further research and understanding of the mechanisms involved.

Our study brings a novel insight into the anti-arrhythmic potential of the border zone innervation based on the effect of beta-adrenergic stimulation on alternans severity. Alternans is an abnormal oscillation of action potential duration and calcium subsystem, typically formed at fast heart rates. Spatially heterogeneous alternans has been clearly implicated in arrhythmogenesis and patients after myocardial infarction have been shown to be at an increased risk of alternans development.

Using optical mapping of rat Langendorff hearts with healed myocardial infarction, we show that the infarct border zone is more prone to alternans formation at slower heart rates compared to the noninfarcted myocardium, providing a pro-arrhythmic heterogeneous substrate. Furthermore, we show that perfusion of the leading sympathetic neurotransmitter norepinephrine considerably attenuates alternans in both normal zone and border zone, reducing electrophysiological heterogeneity, acting anti-arrhythmically, and this effect is blocked by the beta-blocker metoprolol. Using a computer model of a ventricular myocyte (both from normal and border zone) with controllable degree of sympathetic stimulation, we determine a mechanism of the anti-alternans effect, linking it to the modification of dynamics of calcium release from sarcoplasmic reticulum. Our results thus reinforce and at least partially explain the recent human clinical studies showing the surprising association between border zone denervation and future risk of arrhythmia.

Poster – 48

Mechanistic basis for a novel enzyme in genome stability
by Benjamin Foster

Genetic information encoded in DNA needs to be accurately duplicated and passed on to daughter cells in order for an organism to survive and propagate. To maintain the integrity of the genome, cells utilise a range of signalling pathways in a process termed DNA repair. DNA repair proteins act to sense different types of DNA lesions and signal effective repair of the damage. Crucially, defects within DNA repair pathways lead to a range of pathologies and cancers, so understanding the mechanisms of action is of fundamental importance. An important aspect of the DNA damage response (DDR) is the post-translational modification (PTM) of proteins at sites of DNA damage to regulate their activity or localisation, and ubiquitin is one such PTM. ZUP1 (zinc finger-containing ubiquitin peptidase 1) was recently discovered as a novel DUB and was proposed to be involved in the DDR at damaged replication forks. The chain specificity and efficient DUB activity of the C78 peptidase domain of ZUP1 for K63-linked poly-ubiquitin chains are brought about by several ubiquitin-binding domains including a motif interacting with ubiquitin (MIU), ZUP1 helical arm (ZHA) and a ubiquitin-binding zinc finger (UBZ). To gain a deeper understanding of ZUP1 function, I will be investigating other key domains within ZUP1 and how they contribute to ZUP1 activity using a biochemical approach. Presented here are preliminary results and ideas for progressing the project forward.