SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Dunn School Postdoc Symposium 2023

Friday the 17th March

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University of Oxford

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Arranged by

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Schedule

8:30 9:00	Registration / Poster set-up
9:00 9:15	Welcome
	Session 1 **Chair: QianLin Lee**
9:15 9:30	**Isaac Wong** (Raff lab) Mitotic centrosomes are assembled upon co-existing solid- and liquid-like scaffolds
9:30 9:45	**Margaret Varga** (Maiden Lab) Interactive visual analysis of Neisseria Meningitidis
9:45 9:50	**Simon Bush** (Evident Olympus) Advancements in high throughput slide scanning
9:50 10:05	**Yoel Klug** (Carvalho Lab) Mechanism of lipid droplet formation by the yeast sei1/ldb16 seipin complex
10:05 10:20	**Iqbal Dulloo** (Freeman Lab) Cleavage of the pseudoprotease iRhom2 by the signal peptidase complex reveals an ER-to-nucleus signalling pathway
10:20 10:50	Coffee/Tea break
	Session 2 **Chair: Nada Mohamad**
10:50 11:05	**Rene Baerentsen** (Tang Lab) Neisseria spp. Type VI Secretion System immunity proteins are distributed in commensal members of the oropharyngeal flora
11:05 11:20	**Inna Zukher** (Proudfoot Lab) Location matters: Targeted transcription elongation blockage by dCas9
11:20 11:25	**iotaSciences**
11:25 12:10	Invited Speaker: **Prof. Adele Marston** Functional organisation of pericentromeres in mitosis and meiosis
12:10 14:00	Lunch & Poster presentation

Session 3
Chair: Yuichi Tsuchiya

14:00 – 14:15 **Vicky Stancheva** (Sanyal Lab)
Analysing the contact site rearrangement during flavivirus infection

14:15 – 14:30 **Sam Washer** (James Lab)
Genome-wide CRISPR knockout screening to identify key regulators of phagocytosis in induced pluripotent stem cell derived microglia

14:30 – 14:35 **Merck**

14:35 – 14:50 **Rebecca Smith** (Ahel Lab)
HPF1-dependent histone ADP-ribosylation triggers chromatin relaxation to promote the recruitment of repair factors at sites of DNA damage

14:50 – 15:05 **Ecco Staller** (Fodor Lab)
Interaction of human ANP32B with influenza A virus polymerase provides insight into viral transcription and replication

15:05 – 15:40 Announcements & Coffee break

Session 4
Chair: Domagoj Baretic

15:40 – 15:55 **Alexandra Bisia** (Robertson Lab)
Manipulating Eomes activity in the early mouse embryo using a degron-based system

15:55 – 16:10 **Qilin Long** (Gullerova lab)
The role of SOSS complex in transcription regulation at double strand breaks

16:10 – 16:15 **Manni Uppal** (Cambridge Biosciences)
CellDrop and Puffin Packaging

16:15 – 17:00 Invited Speaker: **Prof. Jacques Neefjes**
Lysosomal movement and interactions within the crowded cell

17:00 – 17:15 Closing remarks

17.15 – 18.00 Drinks reception & Prize presentation

List of Posters

1. **Frances Colles** (Maiden lab)

A One Health perspective for reducing antimicrobial resistant Campylobacter in the food chain

2. **Alan Wainman** (Raff lab)

The dynamics of centriole (dis)engagement in Drosophila

3. **Ivana Suchánková** (Walsh lab)

Designing an IL-6 biosensor for the early detection of sepsis

4. **Evangelos Mourkas** (Sheppard lab)

Urbanization and human proximity contribute to the spread of antimicrobial resistance in wild birds

5. **Saroj Saurya** (Raff lab)

Green initiatives at Dunn School

6. **Daniela Vitali** (Carvalho lab)

The derlin Dfm1 promotes retrotranslocation of folded protein domains from the endoplasmic reticulum

7. **Shaked Ashkenazi** (Freeman lab)

New Roles for iRhom2 in Innate Immunity

8. **Anne Hedegaard** (James lab)

Investigating BIN1 involvement in tau handling and extracellular vesicle secretion in iPS-microglia

9. **Zsofia Novak** (Raff lab)

Unravelling centriole-to-centrosome conversion in Drosophila

10. **Michael van de Weijer** (Carvalho lab)

Tapasin is a substrate for the RNF185-Membralin ERAD complex

11. **Lucie Kafkova** (Freeman lab)

RHBDL4: life beyond ERAD?

12. **Franz Wendler** (Baena-Lopez lab)

Caspase regulation of kinesin-dependent beta-integrin trafficking for correct development of the Drosophila neuromuscular junction

13. **Zhe Ji** (Carvalho lab)

Mechanism of CTDNEP1 Phosphatase Regulated Degradation of Inner Nuclear Membrane Protein SUN2

14. **Deeksha Munnur** (Sanyal lab)

Studying replication organelles of Sars-CoV-2

15. **Yuichi Tsuchiya** (Carvalho lab)

Quality control of OXPHOS proteins in the ER

16. **Chia-Chun Chang** (Raff lab)

Reconstituting Centrosome Assembly in Drosophila Embryos on Synthetic Beads

Talk Abstracts

Isaac Wong (Raff lab)

Mitotic centrosomes are assembled upon co-existing solid- and liquid-like scaffolds

Mitotic centrosomes are formed when centrioles recruit pericentriolar material (PCM) around themselves. The PCM comprises several hundred proteins, and its physical nature is hotly debated. In flies, Spd-2/CEP192 recruits Polo/PLK-1 and Cnn/CDK5RAP2 (fly/human nomenclature) to the centriole, and these form a solid-like scaffold that recruits PCM clients. Here we show that Spd-2 also recruits a liquid-like scaffold to centrioles that depends on Aurora A and TACC and is analogous to the liquid-like spindle domain (LISD) in mammalian acentrosomal meiotic spindles. The Polo/Cnn and Aurora A/TACC scaffolds can assemble and recruit PCM clients independently of one another, but centrosome function is severely perturbed in the absence of either scaffold. Thus, mitotic centrosomes in flies assemble upon co-existing solid- and liquid-like scaffolds organised by Spd-2/CEP192 and either Polo/PLK1 or Aurora A, respectively.

Margaret Varga (Maiden Lab)

Interactive visual analysis of Neisseria Meningitidis

Bacterial meningitis is an infectious disease of the brain that occurs worldwide and is a major public health challenge. A leading cause of this often-fatal disease is the bacterium Neisseria meningitidis, also called the meningococcus. Genomics is having a transformational impact on medicine, enabling advances in confirming diagnosis, analysis and prediction of anti-microbial resistance, development and assessment of vaccines etc. PubMLST.org is a major open-access genomics reference database. It is an integrated collection of databases consisting of phenotype and provenance data (metadata) linked to nucleotide sequence data up to and including genome assemblies, for molecular typing of many bacterial species. The large size and complexity of these rich genomic databases mean that it is challenging to gain insight into, and understanding of, the information they contain.

Visual Analytics (VA) provides an effective means of extracting from data, interactively exploring and analysing, information so as to enable users to gain insight into, and understanding from, data; and thus, to develop informative contextual views of the situation. Among the research challenges in designing interactive VA systems is working out how to extract, analyse and enable the effective exploration of information contained within the data. It is especially challenging to transform inherently non-visual data, such as genomic meningitis data, into a natural, intuitive, and easily accessible visual form suitable for a wide range of users.

This presentation discusses our visual analytics and visualization work exploring and analysing Neisseria meningitidis data from PubMLST.

Yoel Klug (Carvalho Lab)

Mechanism of lipid droplet formation by the yeast sei1/ldb16 seipin complex

Lipid droplets (LDs) are universal lipid storage organelles with a core of neutral lipids, such as triacylglycerols, surrounded by a phospholipid monolayer. This unique architecture is generated during LD biogenesis at endoplasmic reticulum (ER) sites marked by Seipin, a conserved membrane protein mutated in lipodystrophy. Here structural, biochemical and molecular dynamics simulation approaches reveal the mechanism of LD formation by the yeast Seipin Sei1 and its membrane partner Ldb16. We show that Sei1 luminal domain assembles a homooligomeric ring, which, in contrast to other Seipins, is unable to concentrate triacylglycerol. Instead, Sei1 positions Ldb16, which concentrates triacylglycerol within the Sei1 ring through critical hydroxyl residues. Triacylglycerol recruitment to the complex is further promoted by Sei1 transmembrane segments, which also control Ldb16 stability. Thus, we propose that LD assembly by the Sei1/Ldb16 complex, and likely other Seipins, requires sequential triacylglycerol-concentrating steps via distinct elements in the ER membrane and lumen.

Iqbal Dulloo (Freeman Lab)

Cleavage of the pseudoprotease iRhom2 by the signal peptidase complex reveals an ER-to-nucleus signalling pathway

iRhoms are pseudoprotease members of the rhomboid-like superfamily and are cardinal regulators of inflammatory and growth factor signalling; they function primarily by recognising transmembrane domains (TMDs) of their clients. Here we report an unexpected, and mechanistically distinct, nuclear function of iRhoms. iRhom2 is a non-canonical substrate of signal peptidase complex (SPC), the protease that removes signal peptides from secreted proteins. Cleavage of iRhom2 at the juxtamembrane region of TMD1 generates an N-terminal fragment that enters the nucleus and modifies the cellular transcriptome. We observed elevated nuclear iRhom2 in skin biopsies of patients with psoriasis and tylosis with oesophageal cancer (TOC), and increased SPC-mediated iRhom2 cleavage in a psoriasis cellular model. This work highlights the pathophysiological significance of this SPC-dependent ER-to-nucleus signalling pathway, and is the first example of a rhomboid-like protein that mediates protease-regulated nuclear signalling.

Rene Baerentsen (Tang Lab)

Neisseria spp. Type VI Secretion System immunity proteins are distributed in commensal members of the oropharyngeal flora

Type 6 Secretion Systems (T6SSs) are a contact-dependent mechanism for bacteria to deliver toxic effectors; toxins have cognate immunity proteins, which prevent self-intoxication, but can also confer protection against T6SS attack. We have previously identified a plasmid encoded T6SS with effector/immunity pairs in Neisseria cinerea, which colonises the human upper respiratory tract. Neisseria spp. are competent for DNA uptake, and pathogens such as Neisseria gonorrhoeae have been shown to acquire antimicrobial resistance genes from commensals; acquisition of immunity genes would allow them to evade T6SS attack. Despite this, the distribution of T6SS immunity genes and the targets of Neisseria T6SS are unknown.

We interrogated NCBI RefSeq, NCBI wgs, and eHOMD databases for homologues of immunity proteins encoded by N. cinerea and show their distribution in both Gram positive and Gram negative commensals of the upper respiratory microbiome (including Streptococcus and Haemophilus). Using the nine immunity genes we performed a neighbourhood analysis to identify 40+ different immunity genes in commensal Neisseria, several being novel folds. No homologues of these were detected in 4000+ genomes of pathogenic Neisseria.

We show that similar immunity genes are present among different commensal Neisseria species and are found in other species of the human upper respiratory microbiome. The absence of immunity genes in pathogenic Neisseria indicates the usage of alternative ways of avoiding T6SS attacks. Furthermore, the identification of new immunity protein families revealed several associated toxin domains, many of which have uncharacterised activities.

Inna Zukher (Proudfoot Lab)

Location matters: Targeted transcription elongation blockage by dCas9

We describe differential molecular consequences of non-cleaving Cas9 (dCas9) placement at varied positions within active transcription units of protein coding genes. We show that dCas9 mediates precise transcriptional pausing, which perturbs the normal Pol II elongation process, induces transcription termination and may alter alternative polyadenylation. We find that dCas9 induced pausing has no effect chromatin associated repressive marks, such as H3K9 di- or trimethylation, suggesting that histone methylation as seen for some terminators is an indirect effect. We explain the asymmetry of dCas9 effects by demonstrating that only binding to antisense target sites induces transcriptional pausing. Binding to sense sites has minimal effects on elongation complex progression and so provides a neutral approach to recruit chromatin modifiers and other effector domains to specific gene regions. In essence we expand molecular understanding of dCas9 effects and in so doing provide new information on co-transcriptional pre-mRNA processing.

Vicky Stancheva (Sanyal Lab)

Analysing the contact site rearrangement during flavivirus infection

Flaviviruses are globally distributed pathogens with high associated morbidity and mortality, which lack effective control measures. Our current knowledge on the underpinning molecular mechanisms of their intracellular lifecycle is limited. Typical of all (+)RNA viruses, flaviviruses induce large rearrangements in the host's endomembrane network to generate replication organelles (ROs). RO formation depends on host organelles including the endoplasmic reticulum, mitochondria and lipid droplets. These are connected through membrane contact sites, which regulate organelle homeostasis and immune signalling, and are altered during infection. The molecular mechanism of contact site remodelling and its significance in flavivirus infection and associated immune responses, however, is understudied. Here I present my work on establishing a versatile proximity-based labelling system to map contact site changes during flavivirus infection. This is corroborated with data showing the involvement of contact site proteins during flavivirus infection.

Sam Washer (James Lab)

Genome-wide CRISPR knockout screening to identify key regulators of phagocytosis in induced pluripotent stem cell derived microglia

Background:
A whole genome knockout CRISPR screen in human induced pluripotent stem cell (hiPSC) derived microglia was undertaken to identify regulators of phagocytosis. The secondary objective was to cross reference these genes to Alzheimer's Disease genome-wide association studies to provide potential therapeutic targets for drug development.

Method:
HiPSC-derived microglia precursor cells were transduced with the TKOv3 genome-wide CRISPR/Cas9 knockout library and differentiated for two weeks to microglia in our recently published media. hiPSC-microglia were then fed dead GFP/mCherry fluorescent labelled SH-SY5Y neuronal cells as phagocytic meal. Phagocytosis proceeded for 6 hours before washing away non-phagocytosed SH-SY5Y, harvesting and fixing the hiPSC-microglia. hiPSC-microglia were sorted into high and low levels of phagocytosis by fluorescent activated cell sorting (FACS) for single mCherry-positive cells as GFP is quenched due to a low pH environment. Genomic DNA was extracted from sorted cell populations and the integrated guide RNA were sequenced by Illumina Novoseq.

Result:
Microglia were successfully transduced at a multiplicity of infection at 0.7 to maximize single guide integration. Successful phagocytosis was observed via FACS, with over 60% of hiPSC-microglia phagocytosing dead neurons after 6 hours. Four populations of varying levels of phagocytosis were harvested and genomic DNA extracted and sequenced.

Conclusion:
We have developed a pipeline for the first successful genome-wide CRISPR knockout screening in hiPSC-microglia and have used this to identify regulators of phagocytosis.

Rebecca Smith (Ahel Lab)

HPF1-dependent histone ADP-ribosylation triggers chromatin relaxation to promote the recruitment of repair factors at sites of DNA damage

PARP1 activity is regulated by its cofactor HPF1. The complex formed by HPF1 and PARP1 catalyses ADP-ribosylation of serine residues of proteins nearby DNA breaks, mainly PARP1 and histones. However, the impact of HPF1 on DNA repair regulated by PARP1 remains unclear. In this study we show that HPF1 controls histone ADP-ribosylation in the vicinity of the DNA breaks by regulating both the number and the length of the ADP-ribose chains. Furthermore, we demonstrate that HPF1-dependent histone ADP-ribosylation triggers the rapid unfolding of chromatin, facilitating access to DNA at sites of damage. This process promotes the assembly of both the homologous recombination and non-homologous end joining repair machineries. Altogether, our data highlight the key roles played by the PARP1/HPF1 complex in controlling ADP-ribosylation signaling as well as the conformation of the damaged chromatin at early stages of the DNA damage response.

Ecco Staller (Fodor Lab)

Interaction of human ANP32B with influenza A virus polymerase provides insight into viral transcription and replication

Influenza A virus (IAV) relies on host factors for transcription (RNA polymerase II) and replication (ANP32). Both processes are carried out by the viral RNA-dependent RNA polymerase (FluPol) in the cell nucleus. During transcription, FluPol interacts with the carboxy terminal domain (CTD) of RNA Pol II, enabling cap snatching. ANP32 proteins mediate the formation of a dimeric FluPol complex that acts as a replication platform. Previous work from the Fodor and Grimes laboratories shows how ANP32A interacts with influenza C virus polymerase, but structures of the more medically relevant IAV polymerase in complex with ANP32 have been elusive. Here we present a structure of a monomeric highly pathogenic H5N1 IAV polymerase with human ANP32B. The binding interface overlaps with the known RNA Pol II CTD interaction site, suggesting an additional role for ANP32B in capturing newly synthesised FluPol as it enters the cell nucleus. Binding of FluPol to ANP32B could facilitate a switch from viral transcription to replication which occurs when sufficient viral proteins have accumulated.

Alexandra Bisia (Robertson Lab)

Manipulating Eomes activity in the early mouse embryo using a degron-based system

Gastrulation is the developmental process by which the three germ layers of the vertebrate embryo (ectoderm, mesoderm, and definitive endoderm) are specified. T-box transcription factor Eomesodermin (Eomes) is essential both to initiate the process of gastrulation itself, as well as to specify the definitive endoderm layer. However, as Eomes expression during gastrulation is brief and very dynamic, it is challenging to dissect its roles in these processes in vivo. We have designed a degron-based Eomes allele, which allows us to inducibly degrade Eomes protein by administration of small molecule dTAG-13, and generated a mouse line harbouring this allele. Homozygous Eomes-degron mice are viable and fertile, and the allele is functional as shown by ex vivo dTAG-13 embryo treatment. However, dTAG-13 treatment in vivo results in inconsistent Eomes protein depletion, and protein recovery is rapid. Nevertheless, we observe anterior midline defects at mid-gestation, consistent with loss of Eomes function. Application of more sophisticated, recently-developed ex vivo embryo culture methods permitting normal development over several days, in combination with the degron system, will enable us to further elucidate Eomes' functions during gastrulation at high temporal resolution.

Qilin Long (Gullerova lab)

The role of SOSS complex in transcription regulation at double strand breaks

Cells developed comprehensive DNA damage response (DDR) pathways to safeguard genome fidelity. The most detrimental type of DNA damage is DNA double-stranded breaks (DSBs), whose efficient and timely repair requires transcription and activity of kinases. However, how the transcription cycle at DSBs is regulated remains unelusive. Here, we show how sensor of single-strand DNA binding (SOSS) complexes assemble and demonstrated their role in RNA polymerase II (RNAPII) regulation at DSBs. Specifically, damage-activated tyrosine kinase, c-Abl, phosphorylates hSSB1 at Tyr102 and Tyr115, which is required for its association with INTS3 and c9orf80 to form trimeric SOSS complex. The trimeric SOSS complex preferentially binds to R-loop structures formed at DSBs and promotes liquid-liquid phase separation. The phase-separated SOSS complex then serves as a scaffold for phosphorylated RNAPII C-terminal domain (CTD) to stimulate transcription. To complete the transcriptional cycle, INTS6 binds to the c-terminus of INTS3, forming a tetrameric SOSS complex, which further recruits PP2A to dephosphorylate CTD of RNAPII and resolves the phase-separated condensates, facilitating efficient transcription termination at DSBs. We propose a dynamic model of the SOSS complex in modulating the transcription cycle at DSBs.

Poster Abstracts

1. **Frances Colles** (Maiden lab)

A One Health perspective for reducing antimicrobial resistant Campylobacter in the food chain

Introduction: Antibiotic use on UK chicken meat farms has fallen by 76% overall and 97.3% for critically important antibiotics between 2012-2019, yet we see fluoroquinolone resistance continue to rise for Campylobacter. We focus on this prominent cause of human gastroenteritis, for which contaminated chicken meat is a major source of infection, due to it's ubiquitous nature, potential to recombine with other gut bacteria and increasing rates of antimicrobial resistance. The organism is on the WHO watch list for antibiotic resistant organisms.

Methods: Using data from our own isolate collection and those on the PubMLST database (https://pubmlst.org), we investigated the epidemiology of antimicrobial resistance. We combined these studies with the development of culture-independent methods to trace the source of Campylobacter for commercially reared chicken flocks. Chicken welfare was assessed by optical flow analysis of flock behaviour, alongside industry production and health data.

Results: There was evidence of clonal expansion of poultry associated fluoroquinolone Campylobacter lineages, with some showing global distribution. Our work with the commercial chicken industry suggests culturable levels of Campylobacter may be indicative of gut dysbiosis and sub-optimal welfare within chicken flocks, potentially leading to increased risk of antibiotic intervention.

Conclusion and future directions: Combining detailed genomic, microbiome and animal welfare studies such as this allows us to more precisely understand how flock management can be optimised to produce flocks that are more resilient to infectious disease and reduce the need for antibiotic use.

2. **Alan Wainman** (Raff lab)

The dynamics of centriole (dis)engagement in Drosophila

Centrioles are important cellular organelles involved in the formation of both cilia and centrosomes. It is therefore not surprising that their dysfunction may lead to a variety of human pathologies.

Centrioles are dynamic structures that change throughout the cell cycle. At the beginning of a cycle, each centrosome contains one centriole, from which a “daughter” centriole grows in an orthogonal orientation during S phase. At the end of the cell cycle the centriole pair separates, and the daughter centriole is then able to mature and nucleate its own daughter.

The separation of daughter centriole from its mother is a process known as disengagement and surprisingly poorly studied. One reason for this is that the daughter and mother centriole are below the resolution limit (defined by Abbe) and therefore appears as a single dot on a standard microscope.

Here we combine super-resolution spinning disc confocal microscopy and tracking image analysis, to follow the position of centrioles in live embryos and reveal the dynamics of mother-daughter engagement during the cell cycle.

3. **Ivana Suchánková** (Walsh lab)

Designing an IL-6 biosensor for the early detection of sepsis

Sepsis is a life-threatening condition that accounts for 20% of global deaths. The current diagnostic routine of sepsis is acutely slow and is based on non-specific symptoms. Interleukin 6(IL-6) is a proinflammatory cytokine that plays a vital role in the body's immune response. Among other molecules, Il-6 was found to be a potent biomarker for detecting early-stage sepsis. Our team developed a biosensor prototype for the rapid detection of IL-6 in blood plasma. To achieve this, we successfully adapted an electrochemical aptamer-based detection principle. We immobilized Il-6-specific aptamers to recognize and bind IL-6 from the patient's plasma sample. Upon binding to IL-6, the aptamers change their conformation. The conformation change allows us to measure IL-6 concentration using electrochemical impedance spectroscopy. We can measure the concentration of Il-6 within the dynamic range of 1-2000 pg/mL of Il-6 in blood plasma under 5 minutes. Our device scored in the top three at the SensUs international biosensor competition in Eindhoven.

4. **Evangelos Mourkas** (Sheppard lab)

Urbanization and human proximity contribute to the spread of antimicrobial resistance in wild birds

Campylobacter jejuni is a multi-host bacterial pathogen responsible for human diarrheal diseases and is frequently isolated from wild birds. While urbanization intensifies worldwide, more wild birds become adapted to urban environments, potentially increasing the risk of zoonotic pathogen transmission. The impact this is having on the microbiome of wild bird populations has not been characterised.

We combine microbial genomics techniques with an understanding of ecological and life-history traits to interrogate a large collection of Campylobacter genomes sampled from wild bird species. Our collection of more than 700 C. jejuni genomes, from 30 different wild bird species, were screened for the presence of antimicrobial resistance (AMR) genetic determinants to better understand the spread of antimicrobial resistance and potential for emergence of novel zoonotic pathogens. Spatial overlap analysis was used to measure bird proximity to urban habitats and phylogenetic generalized least squares models were used to detect associations between lineages, AMR, bird life-history traits and ecological variables.

C. jejuni populations were associated with either one (specialist) or multiple (generalist) wild bird species. Corvidae and Turdidae bird families demonstrated the greatest niche overlap with human habitation and harboured multiple C. jejuni lineages, that were often AMR. A positive correlation between proximity to urbanization and both lineage diversity and carriage of AMR determinants was noted for all bird species.

Understanding the epidemiological dynamics of zoonotic transmission and network of human-animal interactions will better inform us on the spread of antimicrobial resistance and help guide effective surveillance and control measures.

5. Saroj Saurya (Raff lab)

Green initiatives at Dunn School

Climate change is an emergency, everyone including Scientists and laboratories across the world should take drastic measures to reduce their carbon footprint and prevent future climate disasters. For the last three years, the Dunn School green group along with the University of Oxford Environmental sustainability team is working hard to make the Dunn School Green as much as possible. Raff lab has been a pilot lab for various green initiatives that were rolled out throughout the department. Here I describe what a lab, institute, and University can do to reduce their Carbon impact so that we can save our beautiful Earth for future generations to come.

6. **Daniela Vitali** (Carvalho lab)

The derlin Dfm1 promotes retrotranslocation of folded protein domains from the endoplasmic reticulum

Endoplasmic reticulum (ER) proteins are degraded by proteasomes in the cytosol through ER-associated degradation (ERAD). This process involves retrotranslocation of substrates across the ER membrane, their ubiquitination and subsequent membrane extraction by the Cdc48/Npl4/Ufd1 ATPase complex prior delivery to proteasomes for degradation. Recently a mechanism for the retrotranslocation of misfolded substrates by the Hrd1 ubiquitin ligase complex was described. However, how membrane substrates with folded luminal domains are retrotranslocated remains unknown. Here, we identify Dfm1 as an essential membrane component for the retrotranslocation of proteins with folded luminal domains. Both Dfm1 intramembrane rhomboid-like features and the cytosolic Cdc48-binding domains are essential for substrate retrotranslocation. Additionally, substrate processing by Dfm1 and Cdc48 complex requires the ubiquitin shuttle factors Rad23/Dsk2 and the multi-ubiquitination enzyme Ufd2. We further show that this process is conserved in human cells, where Derlins contribute to the degradation of proteins with large and folded luminal domain. Our findings suggest a pathway in which a series of ubiquitin modifying factors recruit Dfm1 to resolve retrotranslocation intermediates due to the presence of a folded luminal domain.

7. **Shaked Ashkenazi** (Freeman lab)

New Roles for iRhom2 in Innate Immunity

The pseudoprotease iRhom2 is a member of the rhomboid superfamily and is conserved from insects to humans. Previous work revealed that iRhom2 plays an important role in the regulation of innate immunity, primarily the trafficking of ADAM17 and the shedding of TNFα and its receptor. In order to further explore the role of iRhom2 in the immune landscape, particularly during the early stages of the response to infections, we extracted bone marrow-derived macrophages from wild-type (WT) and iRhom2 knock-out (iR2KO) mice, and treated them with the secondary messenger 2'3'-cGAMP to simulate an infection with DNA viruses. We then performed RNA sequencing.

Preliminary results demonstrate a profound difference between WT and iR2KO cells, showing a significant basal downregulation of many innate immune genes, such as interleukins and their receptors, TNFα, etc. In addition, the iR2KO macrophages exhibited expression changes in genes associated with chemotaxis.

While these results reinforce previously published findings that the absence of iRhom2 confers immunodeficiency and susceptibility to infections, our data also reveals that iRhom2 is involved in the downregulation of a subset of innate immunity genes, most strikingly, type I interferon and interferon-stimulated genes (ISGs), in addition to certain TLRs, cytokines and chemokines. Our observations demonstrate that iRhom2 is involved in the selective regulation of immune-related genes in macrophages, whereby it is linked with upregulation of pro-inflammatory genes and downregulation of type I interferon genes.

Overall, our results indicate the existence of additional functions of iRhom2 in innate immunity, independent of its regulatory function of TNFα shedding.

8. **Anne Hedegaard** (James lab)

Investigating BIN1 involvement in tau handling and extracellular vesicle secretion in iPS-microglia

Objectives: BIN1 has emerged as a very attractive genetic target for sporadic Alzheimer's Disease (AD), being the next-highest risk factor after APOE. Importantly, BIN1 associates both with risk of disease onset, tau burden and disease progression. Certain BIN1 isoforms have been shown in mouse microglia to contribute to increased incorporation of tau into extracellular vesicles (EVs), and conversely, knocking out BIN1 reduced tau spread via EVs (Crotti et al., 2019). This project utilises human microglia derived from induced Pluripotent Stem Cells (iPSC), to investigate the function of BIN1 in the context of tau processing by microglia, secretion via EVs, and potential implications for neuronal tau pathology.

Methods: We use human iPSC-derived microglia and manipulate their level of BIN1 expression either through knockdown strategies or overexpression of relevant isoforms. Exposing these isogenic microglia with low/endogenous/high BIN1 levels to tau protein then allows assessment of microglial processing of misfolded tau, along with EV production and incorporation of tau into secreted EVs.

Results: We have been developing and testing various tools for BIN1 knockdown and overexpression as well as exploring the sub-cellular localisation of BIN1. We have verified that human iPSC-microglia express various isoforms of BIN1, and that EVs displaying canonical characteristics can be isolated from supernatants.

Conclusions: By carefully characterising BIN1 expression and EV production in human iPSC-microglia, we have laid the groundwork for the next phase of investigations into microglial handling of tau in the context of BIN1.

9. **Zsofia Novak** (Raff lab)

Unravelling centriole-to-centrosome conversion in Drosophila

Centrosomes are the main microtubule organizing centres in animal cells, and they are comprised of a pair of centrioles surrounded by pericentriolar material (PCM). Centrosome duplication is tightly regulated in dividing cells in a way that each inherited mother centriole can only produce a single new daughter centriole per cell cycle. As a part of this control, daughter centrioles do not possess duplicating or PCM-organizing potential until the end of the cycle in which they are assembled, and acquire the license for these processes only once they pass through their first mitosis, through a series of molecular changes that we call centriole-to-centrosome conversion. We have previously shown that Cdk1-dependant Polo recruitment to the core centriolar protein Sas-4 is required for the loading of Asterless (Asl), the key licensing protein for both centriolar duplication and PCM recruitment in Drosophila. In addition, Ana1 also recruits Polo to promote PCM assembly, but not centriole duplication. Here, we explore the molecular network of centriole-to-centrosome conversion in Drosophila embryos, with a particular focus on the interactions and recruitment dependencies of Sas-4, Ana1, Asl and Cep135, a protein previously implicated as one of the most upstream acting proteins within the conversion pathway. Using a combination of live imaging and functional assays in Drosophila embryos as well as general phenotypic analysis of our newly generated cep135 null mutant, we found that Cep135 appears dispensable for centriole duplication in Drosophila, and we also uncovered a novel Cdk1-dependant functional interaction between Sas-4 and Ana1.

10. **Michael van de Weijer** (Carvalho lab)

Tapasin is a substrate for the RNF185-Membralin ERAD complex

Misfolded proteins in the endoplasmic reticulum are eliminated by a process called ER-associated protein degradation (ERAD). This process involves the recognition of misfolded proteins, ubiquitination, their translocation to the cytoplasm, and ultimately delivery to the proteasome for degradation. Recently, we identified a novel ERAD branch comprised of the RNF185-Membralin complex. However, the physiological substrates of the RNF185-Membralin complex remain to be identified. Membralin-deficient mice die perinatally due to acute loss of motor neurons. However, they can be rescued by specific re-expression of Membralin in astrocytes, indicating an essential role for Membralin in this cell type. Therefore, to identify physiological substrates of the RNF185-Membralin complex we used comparative whole cell proteomics in Membralin-deficient mouse astrocytes. This approach specifically identified Tapasin as a substrate for the RNF185-Membralin complex. Tapasin is a component of the peptide-loading complex and is important for recruiting MHC class I molecules to the TAP peptide transporter and loading of MHC class I with high-affinity peptides. Depletion of RNF185-Membralin complex members increases protein levels of both overexpressed and endogenous Tapasin. In addition, Tapasin ubiquitination is strongly reduced in RNF185- and Membralin-depleted cells. Co-immunoprecipitation experiments demonstrate that Tapasin interacts with the RNF185-Membralin complex. Taken together, these findings suggest a potential role for the RNF185-Membralin complex in HLA class I-mediated antigen presentation, the adaptive immune response, and astrocyte function.

11. Lucie Kafkova (Freeman lab)

RHBDL4: life beyond ERAD?

RHBDL4 is an ER-resident serine protease of the rhomboid intramembrane protease family. While its best described function is in ER-associated degradation (ERAD) of aggregation-prone proteins, we have identified novel RHBDL4 substrates that suggest a potential role beyond ERAD. Surprisingly, our substrate trapping screen suggests that RHBDL4 might predominantly cleave non-membrane protein substrates, including multiple ER chaperones and several nuclear proteins not known to localise to the ER. Both groups raise a different set of questions that we are trying to answer.

The nuclear proteins raise a question about the topology of RHBDL4 in the ER membrane. In order for the active site, which typically faces the ER lumen, to encounter these proteins, one of two things has to happen. Either a portion of RHBDL4 is inserted in the opposite orientation, which could allow the cleavage of nuclear proteins not normally present in the ER, or the nuclear proteins could be mislocalised to the ER, leading to their cleavage by RHBDL4. We are currently investigating these possibilities and their potential biological implications.

To investigate the biological implications of the chaperone cleavage, we focus on the finding that the RHBDL4-cleaved fragments of ER chaperones are released into the extracellular space, with several of them cleaved at the C-terminus, effectively removing the ER retention sequence. We are investigating the biological significance of this release of ER chaperone fragments and its potential impact on extracellular protein quality control.

12. Franz Wendler (Baena-Lopez lab)

Caspase regulation of kinesin-dependent beta-integrin trafficking for correct development of the Drosophila neuromuscular junction

Caspase-proteases initiate and execute cell detrimental outcomes ultimately leading to apoptotic cell death. This process is pivotal for tissue development, remodelling, and homeostasis. Here we describe a previously unrecognised role for the Drosophila initiator caspase, Dronc, as a structure-building proponent in the neuromuscular junction (NMJ). During muscle development, correct synapse branching has to keep pace with muscle growth in order to accomplish correct muscle innervation. We found that either reduced or inactive Dronc cause a significant reduction in the number of branches and synaptic boutons at the NMJ. Importantly, these effects are not linked to executioner caspases or Dronc organisers such as Dark protein, thus reinforcing the non-apoptotic nature of Dronc functions at the NMJ. Through proteomic analyses we have identified beta-integrin and the neuron-specific kinesin-motor protein, unc104, as putative Dronc interactors. Intriguingly, these proteins have previously been reported to regulate neuronal branching at the NMJ. Recent immunoprecipitation studies obtained from adult Drosophila expressing any of the studied proteins at physiological levels have confirmed a tripartite interaction between Dronc, beta-integrin, and unc-104. Currently, we are further characterising their interplay at the NMJ from a biochemical perspective. Our studies could reveal for the first time a molecular mechanism by which a caspase carries out a non-apoptotic function by engagement with two previous unknown Dronc-interacting partners.

13. Zhe Ji (Carvalho lab)

Mechanism of CTDNEP1 Phosphatase Regulated Degradation of Inner Nuclear Membrane Protein SUN2

The turnover of the inner nuclear membrane (INM) protein SUN domain-containing protein 2 (SUN2) is through INM-associated degradation pathway (INMAD) and regulated by a phospho-switch mechanism. The major identified phospho-site of SUN2 corresponding to SUN2 degradation is within the nucleoplasmic domain of the protein named site2. C-terminal domain Nuclear Envelope Phosphatase 1 (CTDNEP1) is a known negative regulator for the degradation of SUN2. In the CTDNEP1 knock out (KO) cell lines, the protein level of SUN2 degreased significantly compared with the wildtype cell lines. Previous study has also shown this regulation is related to the lipid sensing by the amphipathic helix (AH) of SUN2. However, the mechanism of how CTDNEP1 regulate SUN2 remains unclear. Here we have preliminary data showing that the regulation of SUN2 homeostasis by CTDNEP1 is not through the activation of Lipin-1 which is the only characterised substrate of CTDNEP1 in mammalian cells so far. Furthermore, by comparison the protein levels of different truncated version of SUN2s in the wild type and CTDNEP1 KO cell lines, we have proved that the site2 and membrane association of SUN2 is sufficient for its responding to the CTNDEP1 regulation. This finding disagrees with the previous study where the AH of SUN2 is required in the CTDNEP1 regulation process. Our data suggests that CTDNEP1 has a direct regulatory role on SUN2, and we will perform in vitro phosphatase assay using recombinant CTDNEP1 on SUN2 to verify this hypothesis. Ultimately, we would like to perform experiments on SUN2 relationships with unresponded protein regulation pathway (UPR), DNA damage and lipid composition changes of nuclear envelope to elucidate the physiological relevance of SUN2 homeostasis.

14. Deeksha Munnur (Sanyal lab)

Studying replication organelles of Sars-CoV-2

Several positive strand RNA viruses form ER-derived structures known as replication organelles which are mainly used for viral RNA synthesis. Coronaviruses form double membrane vesicles (DMVs) that creates a micro-environment which can act as a shield protecting viral RNA against host immune signalling pathway. Non-structural proteins (nsp)-3, 4 and 6 of SARS-CoV-2 are responsible for DMV formation. We aim to understand if any host factors are involved in the DMV formation and also how these nsps 3/4/6 interact to orchestrate the formation of these DMVs.

15. Yuichi Tsuchiya (Carvalho lab)

Quality control of OXPHOS proteins in the ER

Under aerobic conditions, most of ATP is produced by mitochondrial OXPHOS system in normal eukaryotic cells. OXPHOS proteins, encoded by both nuclear and mitochondrial genome, assemble into megadalton complexes in the mitochondrial inner membrane. Pathological and pharmacological stresses perturb the mitochondrial targeting, import and membrane insertion of nuclear genome encoded OXPHOS proteins to lead mistargeting them to other intracellular compartments. Although accumulating evidence suggests that increasing mistargeted proteins into unwanted compartments can cause human diseases, surveillance mechanisms for mistargeted proteins are not fully understood. We found that pharmacological perturbation of membrane insertion of OXPHOS proteins cause ER mistargeting. To further characterize the mistargeting of mitochondrial protein to the ER, we established a split Venus system. This assay and proteomics experiments, show that some complex IV-TMD proteins have strong potentials for mislocalization into the ER, where they become substrates of the ubiquitin-proteasome system. To uncover the molecular mechanism of this process, we did TKOv3 CRISPR screen by using the ER-lumen split Venus system and found that deletions of TOM and TIM complexes genes increase the ER mistargeting and subsets of ERAD machineries play important roles on quality control of ER-mistargeted OXPHOS model substrates. Overall, our studies delineate how cells handle a set of highly hydrophobic proteins to ensure that proper localization and mitochondrial function.

16. Chia-Chun Chang (Raff lab)

Reconstituting Centrosome Assembly in Drosophila Embryos on Synthetic Beads

At the centre of the centrosome is a highly organised structure known as the "centriole." During S phase, centriolar proteins, including Sas6, Ana2, and Sas4, are recruited to the pre-existing mother centriole and assemble the centriolar core structure, known as the cartwheel, to generate a daughter centriole under the control of the master regulator Plk4. The recruitment of Asl, the receptor of Plk4, at the outer layer of the centriole enables the daughter centriole to become a new mother. Whilst the mechanism of centriole assembly is precisely regulated and highly conserved, our previous findings suggest that atypical Ana2-Sas6 complexes could recruit other components and partially resemble functional centrosomes. To explore this further, we have developed a strategy to artificially tether specific centriolar proteins in fruit fly embryos using nanobody-coated microbeads. In embryos that co-express Ana2-GFP and Sas6-GFP, but not either protein alone, the microbeads are capable of organising the microtubule network and generating new centrioles as normal centrosomes would. These findings suggest that the Ana2-Sas6 complex is capable of recruiting crucial centriolar components, including Asl, Plk4, and tubulin, even in an unusual architecture. Further analysis will be carried out to examine the molecular dynamics and protein composition of the Ana2-Sas6 microbeads. This could potentially be further applied to investigate the molecular mechanism of individual steps involved in centrosome assembly.