RNA Club

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Annual Scientific Meeting

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South Cloisters, Room 3.06

University of Exeter, St Luke's Campus, Exeter EX1 2LU

Meeting sponsored by:

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South West UK RNA Club – Annual Scientific Meeting

Agenda

9.00	Registration	
9.45	Welcome and Opening Remarks – Mike Ladomery & Seb Oltean	
ALTERNATIVE SPLICING 1 – Chair: Lorna Harries		
9.50	**Dr. Soma Meran** (Cardiff University)	*'Nuclear hyaluronidase 2 drives alternative splicing of CD44 pre-mRNA to determine profibrotic or antifibrotic cell phenotype'*
10.05	**Dr. Elizabeth Bowler** (University of Exeter)	*'Compounds and signalling mechanisms that regulate VEGF-A_{xxxb} splice site selection'*
10.20	**Simon Uzor** (UWE)	*'Treatment of prostate cancer cells with the CLK1 inhibitor TG003 reduces tumour cell growth and induces apoptosis'*
10.35	**Ling Li** (University of Exeter)	*'Modulation of alternative splicing regulators during EMT in tumour progression'*
10.50	Coffee & Meet the sponsors	
NON-CODING RNAs – Chair: Tim Bowen		
11.20	**Dr. Ioanna Pavlaki** (University of Bath)	*'Paupar LncRNA Promotes KAP1 Dependent Chromatin Changes And Regulates Olfactory Bulb Neurogenesis'*
11.35	**Amy Brook** (Cardiff University)	*'Pathogen-specific local microRNAs in peritoneal dialysis patients with acute peritonitis'*
11.50	**Dr. Daniel Smith** (Cardiff University)	*'Electrochemical Detection of Urinary MicroRNAs'*
12.05	**Serife Ustuner** (University of Bath)	*'PNA Probes and Novel Intercalators for Impedance and Field-Effect based DNA Detection'*
12.20	**Dr. Naveed Anwar** (ThermoFisher Scientific)	*Application Presentation: 'Title TBC'*
12.30	Lunch & Meet the sponsors	
KEYNOTE LECTURE – Chair: Seb Oltean		
13.30	**Dr. Emanuele Buratti** (Group Leader – ICGEB, Trieste, Italy)	*'New insights into RNA-binding proteins and neurodegeneration: the case for TDP-43'*
ALTERNATIVE SPLICING 2 - Chair: Mark Lindsay		
14.30	**Dr. Megan Stevens** (University of Exeter)	*'Identification of novel splicing events in diabetic nephropathy'*
14.45	**Danny Legge** (University of Bristol)	*'Epigenetic inactivation of the epithelial splicing factor ESRP2 in Wilms' tumour'*
15.00	**Chigeru Wodi** (UWE)	*'Targeting the splice factor kinase SRPK1 in leukaemic cells'*
15.15	**Jed Lye** (University of Exeter)	*'Spliceosomics of the ageing brain'*
15.30	Coffee & Meet the sponsors	
RNA BIOLOGY - Chair: Mike Ladomery		
16.00	**Nicola Jeffery** (University of Exeter)	*'The diabetic microenvironment drives conversion to a delta cell-like phenotype in human beta cells'*
16.15	**Ben Jenkins** (University of Exeter)	*'Using RNA interference to dissect cell-cell interactions in a nascent endosymbiosis'*
16.30	**Joshua Eaton** (University of Exeter)	*'Global analysis of RNA polymerase II transcription termination at single nucleotide resolution'*
16.45	Closing Remarks	

Nuclear hyaluronidase 2 drives alternative splicing of *CD44* pre-mRNA to determine profibrotic or antifibrotic cell phenotype

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The cell surface protein CD44 is involved in diverse physiological processes, and its aberrant function is linked to various pathologies such as cancer, immune dysregulation, and fibrosis. The diversity of CD44 biological activity is partly conferred by the generation of distinct CD44 isoforms through alternative splicing. We identified an unexpected function for the ubiquitous hyaluronan-degrading enzyme, hyaluronidase 2 (HYAL2), as a regulator of *CD44* splicing. Standard CD44 is associated with fibrotic disease, and its production is promoted through serine-arginine-rich (SR) protein-mediated exon exclusion. HYAL2 nuclear translocation was stimulated by bone morphogenetic protein 7, which inhibits the myofibroblast phenotype. Nuclear HYAL2 displaced SR proteins from the spliceosome, thus enabling HYAL2, spliceosome components (U1 and U2 small nuclear ribonucleoproteins), and *CD44* pre-mRNA to form a complex. This prevented double-exon splicing and facilitated the inclusion of CD44 exons 11 and 12, which promoted the accumulation of the antifibrotic CD44 isoform CD44v7/8 at the cell surface. These data demonstrate previously undescribed mechanisms regulating *CD44* alternative splicing events that are relevant to the regulation of cellular phenotypes in progressive fibrosis.

Compounds and signalling mechanisms that regulate VEGF-A_{xxxb} splice site selection

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Abnormal angiogenesis is associated with a variety of diseases, including: cancer, various eye diseases such as diabetic retinopathy and age-related macular degeneration (AMD)[1], and several disorders associated with the reproductive system, such as endometriosis and polycystic ovary syndrome[2]. The angiogenesis initiator VEGF-A is differentially spliced to produce multiple isoforms. One particular splicing event that arises from an alternative splice site in exon 8 generates anti-angiogenic VEGF-A_{xxx}b isoforms. These isoforms are functionally opposite to the pro-angiogenic VEGF isoforms that contain the proximal part of exon 8[3].

A screen in our laboratory using the Library of Pharmacologically Active Compounds (LOPAC) in prostate cancer PC3 cells uncovered nine potential compounds that switch splicing to favour the VEGF-A_{xxx}b anti-angiogenic isoform. These compounds have been validated in angiogenesis assays *in vitro* (Matrigel, co-culture of endothelial cells and fibroblasts) and *in vivo* (Matrigel plugs and tumour xenografts).

Previous studies have implicated the splice factors SRSF1 and SRSF6 in determining splice site selection of exon 8 of $VEGF_{165}$. SRSF1 has been associated with proximal splice site selection; and overexpression of SRSF6 increased expression of VEGF-A_{xxx}b isoforms[4]. Furthermore, other studies have shown that inhibition of the splice factor kinase SRPK1 switches splicing of VEGF-A to increase the ratio of the anti-angiogenic VEGF-A_{xxx}b isoforms[5]. Preliminary data shows that some but not all of our compounds activate SR-protein phosphorylation.

Further work aims to uncover the mechanisms that are involved in altering the splicing of VEGF under the influence of the compounds, and could uncover novel targets for future therapy.

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Treatment of prostate cancer cells with the CLK1 inhibitor TG003 reduces tumour cell growth and induces apoptosis

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Prostate cancer (PCa) is the most frequently diagnosed cancer in men and the second leading cause of cancer death. There is a need to identify the molecular genetic processes that underpin prostate cancer and to search for new treatments. Alternative splicing affects over 94% of human genes and is regulated by splice factors and splice factor kinases, the latter including SRPK1 and CLK1. CLK1 is a member of the CDC-like protein kinases and phosphorylates SR proteins in the nucleus, enhancing their availability for pre-mRNA splicing. CLK1 expression is regulated through alternative splicing; skipping of exon 4 or retention of intron 4 results in truncated, inactive CLK1. The aims of this project are to study CLK1 alternative splicing and to explore the effects of targeting the splice factor kinase CLK1 in with a molecular inhibitor, the benzothiazole TG003. Two prostate cancer cell lines (androgen independent PC3 and DU145 cells) were treated for 24-48 hours with varying concentrations of TG003 (10nM -100μM). TG003 treatment altered CLK1's own alternative splicing in a dose dependent manner, suggesting that a feedback loop mechanism contributes to the regulation of CLK1 expression. TG003 treatment suppressed growth and induced apoptosis in PC3 and DU145 cells, as well as decreasing cell migration and invasion. In vivo mouse work with PC3 xenografts showed a significant reduction in tumour growth following intraperitoneal TG003 administration (50□M). In conclusion, we suggest that targeting CLK1 with TG003 may have potential anticancer benefits.

Modulation of alternative splicing regulators during EMT in tumour progression

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The epithelial-mesenchymal transition (EMT), which has been revealed to occur in cancer progression, is a progression by which epithelial cells lose their cell polarization and cell-cell adhesions, and achieve migratory properties and invasiveness to become mesenchymal cells[1]. Epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) are cell-type-specific regulators of transcripts that switch splicing during the EMT[2]. ESRPs drive splicing events of about 200 genes, which give epithelial phenotype. Loss of ESRPs in EMT induces epithelial-to-mesenchymal phenotypic alterations. One of the well-known genes regulated by ESRPs during EMT is the fibroblast growth factor receptor 2 (FGFR2)[3] which has a switch between two mutually exclusive exons – exon8/IIIb and exon9/IIIc[4]. Previous studies have shown ESRPs as master regulators of EMT and underlie EMT during tumour progression[2,5]. As FGFR2 splicing is a sensor of ESRPs activity and EMT, it could be used for understanding the regulation of ESRP in tumors. Using a splicing-sensitive fluorescent reporter based on inclusion/exclusion of FGFR2 exon IIIc we have completed a screen with the LOPAC library (Library of Pharmacologically Active Compounds) to identify compounds that induce exclusion of exon IIIc in FGFR2 and hypothetically block EMT. We have identified several compounds that are able to switch FGFR2 splicing, currently being validated as modulators of epithelial-mesenchymal transitions (e.g they induce expression and junctional localization of E-cadherin), show various activities in functional assays (cell growth and migration) *in vitro*, and one of them siginificant decrease in tumour growth *in vivo*. We have also found that ESRPs overexpression may suppress tumour growth *in vivo* in xenografts of a prostate cancer cell line (PC3).

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Paupar LncRNA Promotes KAP1 Dependent Chromatin Changes And Regulates Olfactory Bulb Neurogenesis

Ioanna Pavlaki[1#], Farah Alammari[2#], Bin Sun[2], Neil Clark[3], Tamara Sirey[3], Sheena Lee[2], Dan J Woodcock[4], Chris P Ponting[3], Francis G Szele[2] and Keith W Vance[1]

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Long non-coding RNAs (lncRNAs) have been implicated in the regulation of central nervous system (CNS) development. However, their roles in vivo as well as the underlying mechanisms of action for the majority of these transcripts remain poorly understood. Paupar is a vertebrate-conserved, CNS-expressed lncRNA that acts to regulate neuroblastoma cell self-renewal and differentiation in vitro by binding and modulating the activity of genome-wide transcriptional regulatory elements. Here, we further examine the role of Paupar and find that it directly binds KAP1, an essential epigenetic regulatory protein, and thereby regulates the expression of shared target genes important for proliferation and neuronal differentiation. Paupar operates as a transcriptional cofactor to promote KAP1 chromatin occupancy in a KRAB-ZNF independent way, as well as H3K9me3 deposition at a subset of distal targets, through the formation of a DNA binding ribonucleoprotein complex. This complex is comprised of Paupar, KAP1 and the PAX6 transcription factor. Paupar-KAP1 genome-wide co-occupancy reveals a 4-fold enrichment of overlap between Paupar and KAP1 bound sequences, the majority of which also appear to associate with PAX6. Furthermore, both Paupar and Kap1 loss of function in vivo disrupts olfactory bulb neurogenesis. These observations provide important conceptual insights into the trans-acting modes of lncRNA-mediated epigenetic regulation, the mechanisms of KAP1 genomic recruitment and identify Paupar and Kap1 as regulators of neurogenesis in vivo.

Pathogen-specific local microRNAs in peritoneal dialysis patients with acute peritonitis

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Peritoneal dialysis (PD) is a daily reality for 250,000 patients worldwide with end-stage kidney failure. However, infection and inflammation-induced damage on the peritoneal membrane remain the major reason for treatment failure. Neither direct identification of the causative pathogen nor current biomarkers are sufficiently accurate or rapid to reliably diagnose infection. We recently showed the local host response evoked by distinct organisms is characteristic enough to identify pathogen-specific 'immune fingerprints' on the day of presentation with acute peritonitis. In these biomarker signatures, infiltrating neutrophils play a prominent but not sufficient role for differential diagnosis.

This study investigates the potential of local microRNAs as biomarkers during acute PD-related peritonitis and aims to define the underlying molecular mechanisms. Our earlier work identified local microRNAs in PD patients associated with the risk of peritoneal membrane fibrosis and technique failure. Here, we recorded pathogen-specific microRNA profiles during acute peritonitis using TaqMan Low Density Arrays and qPCR. As proof-of-concept, we used cell-free samples from patients with well-defined Gram-negative and coagulase-negative *Staphylococcus* infections, representing the two main causative organisms.

Compared to non-infected patients, levels of certain local microRNAs are altered during peritonitis, with some microRNAs increasing (e.g. miR-223) and some decreasing (e.g. miR-31) long before microbiological culture results become available. Similar changes can be detected in a mouse model of bacterial peritonitis. Preliminary *in vitro* studies aimed at defining the cellular sources of diagnostically relevant microRNAs show changes in their levels both intracellularly and released by specific cell types, upon stimulation of primary human mesothelial cells and peritoneal fibroblasts as well as neutrophils and monocytes from human blood, with bacterial extracts or inflammatory stimuli. Our single-centre study shows promise for infection-associated changes in local microRNAs to contribute to pathogen-specific diagnostic immune fingerprints that may guide early treatment and stratification of patients with PD-related peritonitis.

Electrochemical Detection of Urinary MicroRNAs

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Current biomarkers for acute kidney injury (AKI) and diabetic nephropathy (DN) have limited ability to classify disease and stratify patients with respect to disease progression. Using PCR-based methods, we have generated data showing association of urinary microRNAs (miRNAs) with AKI and in DN. To optimise the use of tests based on these associations in the point-of-care environment, we describe here the development of electrochemistry-based methods to detect urinary miRNAs.

We generated a miRNA biosensor by electrical deposition of a naphthalene sulfonic acid derivative onto a glassy carbon electrode (GCE) followed by modification of the sulfonic acid group to a sulfonyl chloride, attachment of a single-stranded amino-labelled DNA probe complementary to the target miRNA, and finally hybridisation with different concentrations of the target miRNA. The electrode surface was analysed through electrochemical coulometry measurements using a ferri/ferrocyanide electrolyte. Our probe detected synthetic miR-21 in buffered solutions to a sensitivity of 20 fM ($2x10^{-14}$ M) as well as being selective down to 1 mismatch. Optimised urinary miRNA detection using this probe compared favourably with RT-qPCR detection.

We have recently been adapting our procedure for use on a set of disposable screen printed electrodes, showing its potential commercial applicability as a disposable biosensor. We have since shown that a significant difference can be seen for DN patient samples for miRs 192 and 126 ($p = 0.034$ and 0.006 respectively.

Work is ongoing looking to optimise the implementation of disposable electrodes and design a new pyrrole based tether for the oligonucleotide probe.

PNA Probes and Novel Intercalators for Impedance and Field-Effect based DNA Detection

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Electrochemical DNA sensors based on the detection of a target DNA by sensing its intrinsic negative charge, can be strongly improved by the use of Peptide Nucleic Acid (PNA) probes. The hybridization of the uncharged PNA probe with the negatively charged DNA target results in a dramatic increase in the charge of the biolayer. The use of PNA has the added advantage of increased specificity and stability. The increase in surface charge can be easily measured with electrochemical impedance spectroscopy (EIS), where variations of charge transfer resistance in the order of 400% can be observed[1]. Non-Faradaic EIS measurements (i.e. without redox markers in solution) are useful for measuring any capacitance changes that occur on the biolayer. On the other hand, both charge variations and capacitance variations can be detected using biologically-sensitive field-effect transistors (BioFETs), where the biolayer is attached to the metal gate of the transistor[2].

In this study we use PNA-DNA redox intercalators to change the surface potential of the electrodes. We have developed and fully characterized an electroactive ferrocenyl PNA-DNA intercalator[3]. The presence of the intercalator allows the direct amperometric detection of the DNA target as well as enhancing the electrochemical impedance of the system. Of significant interest for BioFETs is that the presence of the intercalator induces a change in the formal potential of the system; this in turn modulates the threshold voltage of the transistor providing a sensitive and robust sensing method. An added advantage of the BioFETs is that they can be easily implemented as a monolithically integrated array of sensors providing high-throughput screening of DNA sequences.

We have demonstrated the use of the BioFETs for the detection of *Clostridium difficile*. The bacterium is the primary cause of antibiotic associated diarrhea in humans and is a significant cause of morbidity and mortality. Thus the rapid and accurate identification of this pathogen in clinical samples (such as feces) is a key step in reducing the devastating impact of this disease. We have achieved the BioFET and EIS detection of DNA of *Clostridium difficile* specific toxins extracted via a microwave-based technique[4].

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Keynote Lecture:

New insights into RNA-binding proteins and neurodegeneration: the case for TDP-43

Dr. Emanuele Buratti

ICGEB Trieste, Italy

Since beginning his postdoctoral work, the principal areas of Dr. Buratti's expertise have been the investigation of RNA binding proteins/structure and their influence on translation and alternative splicing processes. In 2001, as part of a research project on Cystic Fibrosis, he identified nuclear protein TDP-43 as a potential CFTR exon 9 pre-mRNA splicing regulator. As a result, in the following years he has performed several basic studies on the biochemical and functional properties of this protein that have been of great advantage to the scientific community when its role in neurodegeneration was first reported in 2006. Since then, Dr. Buratti research has primarily focused on investigating the potential pathophysiological role played by TDP-43 in Frontotemporal Dementia and Amyotrophic Lateral Sclerosis, and in better understanding the functional roles played by this protein (ie. microRNA biogenesis and splicing regulation, shuttling nuclear/cytoplasmic properties, autoregulatory processes, and so forth).

Identification of novel splicing events in diabetic nephropathy

M. Stevens[1] and S. Oltean[1]

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Recent studies have highlighted several splice variants implicated in diabetic nephropathy including VEGF-A_{165}b and esRAGE. It is likely that many more splice variants play a role the pathogenesis of diabetic nephropathy. The present study aimed to identify novel alternative splicing events in cultured glomerular endothelial cells exposed to a diabetic environment.

Conditionally immortalised glomerular endothelial cells were treated with a diabetic environment (30 mM glucose, 1 ng/ml TNFα, 1 ng/ml IL-6 and 100 nM insulin) or an osmotic control (25 mM mannitol + 5 mM glucose) for 1 week. We then carried out RNAseq and analysed changes in overall gene expression (DESeq 2) as well as alternatively spliced gene transcripts (ASprofile) in response to the diabetic environment.

Thus far, we have focused our analysis on the overall expression of splice factors and found 21 splice factors upregulated, and 14 downregulated, after exposure to the diabetic environment. This includes the upregulation of SRSF1, which promotes splicing of the pro-angiogenic VEGF-A_{xxx} isoforms known to be increased in diabetic nephropathy. We are currently validating the expression levels of these splice factors using qRT-PCR. The identification of novel alternative splicing events from the RNAseq data is still ongoing.

Although this study is in the early stages, we found that a diabetic environment resulted in changes in the expression of many splice factors in glomerular endothelial cells, indicating the important and widespread role of alternative splicing in the pathogenesis of diabetic nephropathy.

Epigenetic inactivation of the epithelial splicing factor ESRP2 in Wilms' tumour

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Our genome-wide analysis of DNA methylation had previously identified *ESRP2* as a gene that is frequently hypermethylated in Wilms' tumour (WT; a common childhood kidney cancer), with associated downregulation of expression. *ESRP2* encodes an RNA splicing factor that is essential for epithelial differentiation, a process that is deregulated in WT development.

To investigate the function of ESRP2 in WT development, we generated a WT cell line (WiT49 E200L) with doxycycline (Dox)-inducible expression of ESRP2, along with control cells transfected with empty vector (WiT49 V200). ESRP2 induction following Dox addition was confirmed by PCR and western analysis. Alternative splicing of known ESRP2 targets *FGFR2* and *ENAH* was confirmed by PCR following induction of ESRP2 in E200L cells but was not observed in V200 control cells after Dox addition. Furthermore, colony formation was significantly inhibited by around 50% upon induction of ESRP2. Importantly - and in agreement with colony forming data - cell growth was reduced by around 50% following 6 - 8 days of ESRP2 induction in E200L cells.

Finally, to identify any ESRP2-induced alternative splicing events or gene regulation that may be important in WT development or progression, we performed RNA-Seq analysis in E200L cells treated with DMSO or Dox for 96 hours (this timepoint showed the greatest induction of ESRP2 protein, mRNA and ESRP2-target alternative splicing regulation following Dox addition). Initial analyses show enrichment of genes involved in epithelial-mesenchymal transition among the differentially spliced genes. Data generated from this analysis has the potential to highlight novel alternative splicing events that could potentially be targeted for more effective therapies for Wilms' tumour in the future.

This work was supported by the Little Princess Trust and Children with Cancer

Targeting the splice factor kinase SRPK1 in leukaemic cells

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Aberrant splicing patterns are associated with the development of cancer and so there is growing interest in targeting the machinery of splicing. The serine/arginine splice factor kinase SRPK1 phosphorylates splice factors rich in serine/arginine repeats (RS-domain) which are required for pre-mRNA splicing. Studies have previously shown that targeting SRPK1 in prostate cancer cells drastically reduces tumour growth and is a potential target for treatment (Oltean, S. 2012; Mavrou *et al.* 2015). This study was aimed at investigating the effect of inhibiting SRPK1 in leukaemic cells using SPHINX, a specific small molecule inhibitor both alone and in combination with conventional chemotherapy drugs. Chronic myeloid leukaemia (CML) and Acute myeloid leukaemic (Kasumi-1) cell line were used as models.

SPHINX decreases cell viability and growth in Kasumi-1 but not in K562 cells. Both cell lines showed an increase in caspase3/7 activities at higher concentrations of 10μM SPHINX with Kasumi-1 cells exhibiting a dose dependent response. Capase-9 altered its splicing pattern following treatment with SPHINX. When each cell line was treated with a combination of SPHINX and conventional chemotherapeutic drugs (K562s with imatinib mesylate and Kasumi-1 with azacytidine), increased cell death was observed in the case of Kasumi-1 cells treated with SPHINX and azacytidine. These results indicate that there may be potential benefit in treating some leukemic cells with an SRPK1 inhibitor.

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Spliceosomics of the ageing brain

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Dementia is a devastating age-related disease, and astrocyte dysfunction during ageing has been shown to be heavily implicated in disease progression.

As it has been shown the genes which control the regulation of alternative splicing are those most tightly associated with biological ageing, we investigated potential links between RNA splicing factor levels, associated isoform expression and senescent cellular phenotypes in astrocytes.

We characterised the senescence associated secretory phenotype (SASP) in old and young primary astrocytes, known to be pivotal in the progression of age related cognitive decline. We then compared these changes to specific isoform expression and splicing factor expression. We further intend to compare data regarding temporal changes in mental state of a large human cohort and assess links between apparent age-related decline of cognitive function, and specific isoform expression in the blood, in an attempt to identify a predictive biomarker for dementia.

Our initial results show an almost ubiquitous decrease in splicing factor expression as astrocytes age. In addition, we have seen significant increases in specific cytokines and matrix metallo-proteins characteristic of SASP with increased age.

The diabetic microenvironment drives conversion to a delta cell-like phenotype in human beta cells

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Changes in beta-cell mass occurs in both type 1 (T1D) and type 2 diabetes (T2D), and has been attributed to apoptosis and necrosis. Dedifferentiation and trans-differentiation to other pancreatic endocrine cells has been described in rodents, although this has been relatively poorly explored in humans. We examined the number of insulin, glucagon and somatostatin positive endocrine cells, in pancreatic sections from T1D and T2D patients and matched non-diabetic controls. The effect of the diabetic microenvironment (hyperglycaemia, dyslipidaemia, hypoxia, inflammatory factors) on markers of cell fate and function, in human beta-cells in vitro, was also explored. A significant increase in the number of somatostatin-positive cells was noted in T1D and T2D cases compared to controls using immunohistochemistry. A similar beta to delta-like transition was noted in response to all treatments vitro, and also with the ER-stress inducer tunicamycin. These changes were accompanied by reduced insulin and increased somatostatin mRNA. Profound changes to expression of splicing regulatory proteins and patterns of alternative splicing, for genes with roles in beta-cell fate or function upon treatment were also noted. We observed increases in the expression of a *PAX6* isoform, known to regulate somatostatin expression. Restoration of euglycaemia restored splicing factor expression and alternative splicing patterns to levels comparable with controls. These changes were accompanied by re-establishment of a beta-cell identity, suggesting effects of the diabetic microenvironment are reversible. These data suggest that changes in beta-cell fate may contribute to beta-cell mass loss in both T1D and T2D, but the possibility exists to remedy this phenomenon by restoring normal homeostasis.

Using RNA interference to dissect cell-cell interactions in a nascent endosymbiosis

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Endosymbiosis is a long-established theory relating to the formation of the mitochondria and plastid organelles - a mutual association whereby two became one to form a single integrated system. The photosynthetic endosymbioses between *Paramecium bursaria* and the green algae *Chlorella spp.* represents a nascent facultative endosymbiosis, meaning both host and symbiont are still able to exist and reproduce separately. My discovery of an active RNAi system in *P. bursaria*, a form of gene silencing used extensively in functional analysis, allows systematic characterisation of host genes and functions critical to host-symbiont cell-cell interactions. RNAi cross-talk may be a factor in shaping nascent endosymbiotic interactions and I provide evidence that host genome expression can be altered by RNAi 'collisions' from the endosymbiont using both double-stranded and single-stranded RNA induced RNAi. Interestingly, I discovered that single-stranded RNA only produced an RNAi effect when delivered in the sense orientation to the target gene transcript, and at a greater efficiency than double-stranded RNA induced RNAi, suggesting the potential for preferential strand bias within the RNA-induced silencing complex. From here I seek to further assess how host processing of endosymbiont derived single-stranded RNA has facilitated the shift from transient feeding of microbial food to establishment of the endosymbiont, and whether exposure to endosymbiont-derived sRNA upon digestion is providing a cost to host fitness for killing the endosymbiont, postulating RNAi cross-talk via RNAi 'collisions' as a new factor in the maintenance of this cell-cell interaction.

Global analysis of RNA polymerase II transcription termination at single nucleotide resolution

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Termination is a ubiquitous phase in every transcription cycle but is incompletely understood and a subject of debate. We used gene editing as a new approach to address its mechanism through engineered conditional depletion of the 5′→3′ exonuclease Xrn2 or the polyadenylation signal (PAS) endonuclease CPSF73 (cleavage and polyadenylation specificity factor 73). The ability to rapidly control Xrn2 reveals a clear and general role for it in cotranscriptional degradation of 3′ flanking region RNA and transcriptional termination. This defect is characterized genome-wide at high resolution using mammalian native elongating transcript sequencing (mNET-seq). An Xrn2 effect on termination requires prior RNA cleavage, and we provide evidence for this by showing that catalytically inactive CPSF73 cannot restore termination to cells lacking functional CPSF73. Notably, Xrn2 plays no significant role in either Histone or small nuclear RNA (snRNA) gene termination even though both RNA classes undergo 3′-end cleavage. In sum, efficient termination on most protein-coding genes involves CPSF73-mediated RNA cleavage and cotranscriptional degradation of polymerase-associated RNA by Xrn2. However, as CPSF73 loss caused more extensive readthrough transcription than Xrn2 elimination, it likely plays a more underpinning role in termination.