MOLECULAR BIOLOGY

TIME: 11.30 – 12.30 LOCATION: DOBSON ROOM

MOLECULAR BIOLOGY AND BIOINFORMATICS ANALYSIS OF THE ROLE OF RBMX FAMILY OF PROTEINS IN SPLICING REGULATION

Chileleko Siachisumo, Institute of Genetic Medicine, Newcastle University

RBMX is part of a family of RNA binding proteins that regulate splicing. RBMX is conserved in all invertebrates it has a key role in splicing regulation. Not much is known about RBMX regulation or its targets. RBMX has a homologue called RBMY on the Y chromosome. RBMY and RBMX are two ancient genes that evolved many millions of years ago. Deletion of the Y chromosome where RBMY is located is found in infertile men. RBMX has a several retrotransposons on autosomes. Of note is RBMXL2 on chromosome 12. RBMXL2 has been shown to be involved in male infertility in mice. In our data we have used RNAseq to discover that when RBMX is knocked down there is a mis-regulation of key cell cycle factors. We predict that RBMX may function by direct RNA interactions or protein interactions or both. The motivation of my project is to decipher how RBMX and RBMXL2 regulate RNA targets. This will be done by performing iCLIP to crosslink RNA and protein to map the positions of direct RNA binding sites. These binding sites will be used to predict mechanisms of splicing regulation that I will test using minigenes. The identity of the targets regulated by RBMX should predict what is going wrong with the cells after RBMX siRNA depletion. Already we have identified some important proteins involved in DNA replication and the cell cycle that are affected by RBMX depletion.

THE INHIBITION OF RIBOSOME-ASSOCIATED GTPASES BY (P)PPGPP IN STAPHYLOCOCCUS AUREUS

Daniel Bennison, Molecular Biology and Biotechnology, University of Sheffield

Staphylococcus aureus is a leading cause of invasive human infection, including bacteraemia and a variety of pulmonary infections. During host colonisation, the ability of *S. aureus* to adapt to stressful conditions is paramount to survival and the continuation of infection. During conditions of nutrient limitation, the alarmone (p)ppGpp is produced as the effector of the stringent response, and is known to be involved in cellular adaptation to stress and potentially entry into stationary phase. Genome-wide screening has identified four ribosome-associated GTPases (RA-GTPases), known as RsgA, RbgA, Era and HflX to which (p)ppGpp binds, each of which have been implicated as a cofactor in 70S ribosome assembly. Further study has revealed that when associated with the 70S ribosome, GTPase activity is increased dramatically. However upon (p)ppGpp binding to these RA-GTPases, their activity is inhibited – negatively impacting ribosome assembly and bacterial growth. This mechanism of inhibition of RA-GTPases therefore has potential regarding the design of novel bacteriostatic antimicrobials. Here, the contribution of each of these RA-GTPases to the survival of *S. aureus* in vivo is currently being assessed, in combination with in vitro site-directed mutagenesis, kinetic analyses and X-ray crystallography to determine the molecular mechanism of inhibition by (p)ppGpp. We have shown that as well as (p)ppGpp being a non-hydrolysable GTP analogue, binding to RA-GTPases inhibits association with the ribosomal subunits. in vivo selection of the (p)ppGpp-bound state has revealed a decrease in growth, 70S assembly and translation, outlining RA-GTPases as

major players in stringent response mediated translational control.

RIBOSOME BIOGENESIS AND CANCER; MECHANISM OF P53 ACTIVATION DRIVEN BY DEFECTS IN SMALL RIBOSOMAL SUBUNIT PRODUCTION

Matthew Eastham, Institute for Cell and Molecular Biosciences, Newcastle University

Ribosomes, the protein powerhouses of all cells, are large RNA-protein complexes consisting of two subunits. Due to the fundamental importance of ribosomes in cellular biology, it is unsurprising that defects in their biogenesis are linked to a range of human disorders. Over 20 genetic diseases linked to ribosomal defects are known at present. Notably, defects in the production pathways that generate both the large (LSU) and small (SSU) ribosomal subunits have been linked to a range of cancers. One mechanism underlying this is the role of ribosomes in regulating the tumour suppressor p53, the most commonly mutated gene in human cancer.

It is well recognised that LSU defects activate p53 through an assembly intermediate of its subunit, the 5S-RNP. However, whilst SSU production defects also activate p53 through this LSU assembly intermediate, the mechanism by which it does so is unclear.

Here, we show that p53 activation can occur as early as 6 hours following the introduction of SSU production defects, much earlier than previously reported. Activation of p53 within this time frame is not coupled to mature SSU levels. Using a new system generated to study ribosome biogenesis, inhibiting SSU production resulted in impaired nuclear export of the maturing LSU, as well as blocking late, cytoplasmic steps of LSU production. This suggests that SSU defects activate p53 through stalling the late stages of LSU maturation. Understanding and targeting the mechanism of p53 activation resulting from defective ribosome biogenesis could be a promising therapeutic strategy in preventing disease development.

PXY AND ER RECEPTOR KINASES ENFORCE VASCULAR PATTERNING IN PLANTS

Kristine Bagdassarian, Durham University

Plant vascular tissues transport water and nutrients. Consequently, they are key to plant growth and development. Xylem and phloem are the two main transport tissues. They arise from cell divisions in the meristematic cambium. As plant cells cannot migrate, cell-type specification must occur in a highly organised manner to ensure that tissue integrity is maintained. In Arabidopsis, PXY and ER encode receptor-kinases that regulate this process in the vascular tissue. Loss of the pxy gene disrupts vascular organisation and reduces the number cell divisions. Removal of er from pxy mutants exacerbates these defects. The contribution of PXL and ERL genes, paralogues of er and pxy, to this process is unknown. To address this question, we used classical genetics and genome editing to remove all members of the PXY and ER gene families. In these lines, radial growth and plant cell morphology were investigated using a bespoke MATLAB algorithm and statistical methods. Our results indicate that in the absence of the PXY genes, members of the ER family, and particularly ERL2, function as a compensatory mechanism, maintaining radial growth in the absence of PXY by increasing the size of xylem cells. Plants lacking ER and PXY family receptors failed to transition to true radial growth. Thus, the PXY and ER family of genes coordinate cell size with cell division in vascular tissue which, in turn, maintains hypocotyl size and patterning.