Some Examples of Microscopy-Based Projects in Animal BioSciences

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We present here examples of current microscopy-based projects in Animal Biosciences:

**Functional cellular imaging of immune responses during ageing** \textsuperscript{a},

The immune system is subject to age-related changes that may contribute to increased prevalence and severity of infectious disease in the elderly. We are using 2-photon \textit{in vivo} imaging of ageing mice to investigate dynamic and functional cellular changes. 2-P microscopy permits imaging deep in tissues at cellular/molecular resolution and is suited for imaging lymphoid tissues where key immune responses take place. Dynamic antigen - lymphoid interactions influencing trapping, trafficking and effector response can be observed.

**Primordial Germ Cell Migration in Cultured Chick Embryos** \textsuperscript{b}.

Safeguarding reproduction and fertility depends on understanding normal germ cell development in chickens. Primordial germ cells (PGCs), stem cell precursors for sperm and eggs, differ from most stem cell populations as they migrate extensively from their origin to final niche in the gonad. Aberrant PGC migration is thought to be a cause of germ cell tumours. Mechanisms regulating PGC migration are poorly understood. We used a novel culture system for avian PGCs to study cell migration in vitro and in cultured embryos using a Zeiss cell observer with deconvolution. We have identified novel migration modes in PGCs.

**The role of the TALPID3 gene during cell signalling and early development** \textsuperscript{c}.

The TALPID3 gene (\textit{Ta3}), involved in chick limb development, was mapped in Roslin \cite{1}. \textit{Ta3} is essential for development but protein action is unknown. To understand the role of \textit{Ta3} we utilised a variety of microscopy techniques: (1) brightfield to investigate effect of functional \textit{Ta3} loss, (2) OPT to examine gene expression, (3) live cell imaging of mixed \textit{Ta3} and GFP chick fibroblasts to assess cell behaviour, (4) immunofluorescence to determine \textit{Ta3} localisation / tissue polarity, (5) confocal microscopy to determine \textit{Ta3} protein localisation and (6) TEM/SEM and OMX microscopy (in collaboration with Centre for High Image Processing Dundee). We determined that \textit{Ta3} is a centrosomal protein essential for cilia formation and cell polarity. This places \textit{Ta3} within the class of ‘ciliopathies’, an emerging class of human disorders with severe health repercussions. \cite{1} Genes Dev. 2006 15;20(10): 1365–1377

**SPP1 gene expression in Porcine Reproductive Tissues** \textsuperscript{d}.

The Secreted Phosphoprotein 1 (SPP1) gene has a key role in implantation and maintenance of pregnancy in the pig. We hypothesised that differences in fetal growth may be associated with the effectiveness of conceptus attachment, measured by SPP1 expression. To examine this hypothesis SPP1 expression was assessed, between feto-placental units of different size, by darkfield microscopy assessment of ISH for SPP1 mRNA expression, and by immunohistochemistry in confocal optical slices. These techniques revealed that SPP1 mRNA and protein are located in utero-placental tissues and that there are mRNA differences between tissues and protein differences between size of foetus.

**Phagocytosis in Primary Bovine Macrophages** \textsuperscript{e}.

To investigate the effect of signal regulatory protein beta (SIRPB) gene knockdown on phagocytosis, time-lapse live cell and confocal microscopy were used to monitor phagocytosis of fluorescent beads by primary bovine macrophages. Bead uptake was measured by cytometry, but we also confirmed that beads were engulfed using live cell timelapse. Also, cell membranes were stained with DiD prior to addition of beads and viewed by confocal microscopy, showing beads were internalised. Results suggest that knockdown of SIRPB1 and SIRPB2 does not significantly alter phagocytosis of latex beads.