

Sixth ARK-Genomics Farm Animal Functional Genomics Workshop
September 27th & 28th, 2006
Robinson College,
Cambridge, UK

ABSTRACTS –SPEAKERS**Wednesday September 27th**

13:10 - 14:00 Keynote

Prof Dave Burt – Roslin Institute, UK

Identification of a new molecule in the SHH signaling pathway: Genomics at the cutting edge¹David Burt, ¹Megan Davey, ¹Bob Paton, ²Yili Yin, ²Fiona Bangs, ²Cheryll Tickle

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Talpid3 is a classical chicken mutant with abnormal limb patterning and malformations in other regions of the embryo known to depend on Hedgehog signaling. We combined the ease of manipulating the chicken embryo with the emerging knowledge of its genome to reveal directly the basis of defective Hedgehog signal transduction in talpid3 embryos and to identify the mutation in the talpid3 gene. We demonstrate for the first time that talpid3 is absolutely required for the function of both Gli repressor and activator functions in the intracellular Hedgehog pathway. The talpid3 locus was mapped to chromosome 5 as a frameshift mutation in a KIAA0586 ortholog (ENSGALG00000012025), a gene not previously attributed with any known function. We show a direct causal link between KIAA0586 and the mutant phenotype by rescue experiments. We show that Gli3 processing is abnormal in talpid3 mutant cells but that Gli3 can still translocate to the nucleus. These results suggest that the talpid3 protein operates in the cytoplasm to regulate the activity of both Gli repressor and activator proteins. We are using a number of genomics techniques and resources to understand more about the biological role and molecular function of the talpid3 protein in SHH signaling. Principle amongst these approaches is the use of high density gene expression chips to monitor the expression of all known chicken genes in RNA's extracted from either anterior or posterior regions of stage 24 limbs buds from wild-type and talpid3 mutant embryos. Detailed bioinformatic analyses and whole mount in situ hybridisations have identified clusters of genes that show patterns of gene expression very similar to PTCH and HOXD13 – the classic patterns associated with Gli activator and repressor pathways, respectively. Further work will concentrate on the identification of the cis- and trans-acting factors that co-regulate these genes using yeast-2-hybrid and in vivo reporter assays. Finally, this talk will not only demonstrate what can now be done in chick development but will also show, in a more general sense, how we can exploit the genomic resources and techniques within ARK-Genomics in our research on farm animals and model organisms.

This work was supported by grants from BBSRC, MRC and Wellcome Trust.

14:00 - 14:30 Session 1: Disease & Immunology

Dr Pete Kaiser – Institute of Animal Health, UK

The mechanisms of virus-induced immunosuppression in poultry – the circovirus chicken anaemia virus as a model system.

Pete Kaiser¹, Lisa Rothwell¹, Ruth Bashforth¹, Tuanjun Hu¹, Danny Todd^{2,3}, Alistair Scott^{2,3}, Stathis Giotis^{2,3,4}, Dave Burt⁴ and Liz Glass⁴

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Chicken anaemia virus (CAV) is an immunosuppressive circovirus of increasing prevalence, resulting in economic loss to the poultry industry, and exacerbating the effects of other pathogens. We are investigating the interactions of CAV with its host by profiling the host transcriptome following infection *in vitro* and *in vivo*. This collaboration between IAH, QUB/AFBINI and RI is exploiting a panel of cytokine reagents developed at IAH and a chicken immune-associated microarray developed at RI together with targeted mutants of the three viral genes developed at QUB. Structure/function analysis of CAV genes is determining its pathogenic and immunosuppressive mechanisms and as such is developing CAV as a model for circovirus infections in other species, including mammals.

14:30 - 15:00 Session 1: Disease & Immunology

Dr Emma Borthwick – Roslin Institute, UK

Is there differential gene expression during early BSE infection in cattle white blood cells?

E B Borthwick, S Hawkins, R Talbot, A Brenn, M Groshup, A L Archibald, J L Williams

Differential gene expression within peripheral tissues of BSE infected cattle could provide the means of identification for early BSE infection. The oral route of infection by the infected agent (PrPres) passes from the gastrointestinal system to the CNS via the lymphoreticular and/or vascular systems. Little is known of the effect on gene expression of the host organs during this early infection period; although there is some evidence of decreased levels of erythroid differentiation-related factor (EDRF) in the spleen and erythroid cells in the blood of TSE infected mice. Therefore there is a possibility of using differential gene expression in peripheral tissues as an early diagnostic test for BSE infection. In this project we are investigating the differential gene expression between normal and BSE infected cattle using RNA isolated from spleen and white blood cells. Spleen samples were collected from a DEFRA funded controlled BSE challenge project at VLA in Weybridge using Holstein cattle. Blood samples were collected from a BSE challenge set up at Greifswald, Germany using Simmental cattle. Blood samples were fractionated into PBMs (peripheral blood monocytes). RNA isolated from the spleen and PBM samples was used to probe bovine specific microarrays, using both dual colour array and the Affymetrix Bovine Genome Array. Analysis of the microarray experiments produced gene lists of significantly differentially expressed genes in both spleen and white blood cells. Interestingly there are more down regulated genes during BSE infection compared to up regulated genes against age matched controls. Genes have been classified into GO groupings and some of the gene regulated results have been verified by quantitative PCR. It is possible that the outcome of this project could produce a panel of differential genes in spleen or blood which may be used as part of an early diagnostic test for BSE infection.

15:00 - 15:30 Session 1: Disease & Immunology

Dr Astrid de Greeff – Animal Science Group (ASG), Wageningen, The Netherlands

Expression profiling of udder tissue in response to acute clinical *Streptococcus uberis* infection

Astrid de Greeff¹, Norbert Stockhofe-Zurwieden¹, Lisette Ruuls¹, Hans-Martin Seyfert², Alison Downing³, Richard Talbot³ and Hilde Smith¹

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³ ARK Genomics, Roslin Institute, Roslin, Scotland

Streptococcus uberis (SUB) causes clinical and subclinical mastitis in cattle. Bovine mastitis is economically the most important disease in the dairy industry. To control SUB infections, more insight into the process of pathogenesis is required. Therefore, early host responses of mammary gland tissue after acute SUB infection were studied using microarray analysis and qRT-PCR.

Four lactating heifers were infected intramammary with SUB. The animals were sacrificed as soon as clinical symptoms of mastitis were observed, between 1.5 and 3 days post infection. Tissue samples were sampled from different locations in the udder and both snap frozen for mRNA isolation and formalin fixed for histopathological analysis. The selection of tissue samples used for mRNA isolation was based upon histopathological observations. Both samples in which clear signs of infection were present (infected sample) as well as samples in which signs of infection were absent (uninfected control) were selected.

qRT-PCR analysis was used to study the expression of defensins and toll like receptors (TLR) 2 and 4. In accordance to data presented previously (1), beta defensin expression was strongly induced in SUB infected udder tissue. TLR 2 and 4 were upregulated as well but to a much lesser extent. These data indicate that the innate immune system is upregulated early in the process of SUB pathogenesis.

For microarray analysis a bovine 20K cDNA array was used. Each slide contained duplicates of each spot and a set of control genes. In each microarray experiment an uninfected control sample was compared to a SUB infected sample from the same animal. Samples from different locations in the udder were tested, and all experiments were dye swapped. The data were analyzed using BlueFuse software, and normalized using either R, or the normalization procedure included in BlueFuse. 246 genes were regulated in their expression with a false discovery rate lower than 5% when identified by the infected vs. uninfected comparison. Most of the affected genes were upregulated (197); 49 of the genes were repressed. Homology searches showed that most of the induced genes were associated with the innate immune response. Interestingly, a considerable number of differentially expressed genes found were also identified after analysis of LPS-induced mastitis in a mouse model (2). This clearly indicates the importance of these genes in the early onset of mastitis in general. In the future, a more detailed analysis of the affected genes may result in more insight into the molecular pathways underlying the pathogenesis of SUB mastitis.

1. Goldammer, T., Zerbe, H., Molenaar, A., Schuberth, H.-J., Brunner, T. M., Kata, S. R., Seyfert, H.-M. 2004. Mastitis increases mammary mRNA abundance of β -defensin 5, toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. *Clin. Diagn. Lab. Immunol.* **11**:174-185.
2. Zheng, J., Watson, A.D., and Kerr, D.E. 2006. Genome-wide expression analysis of lipopolysaccharide-induced mastitis in a mouse model. *Infect. Immun.* **74**:1907-1915.

16:00 - 16:30 Session 2: Physiology

Dr Sandrine Dupre – Manchester University, UK

Investigation of transcriptional pathways driving the photoperiodic control of seasonal rhythms in mammals

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The nocturnal melatonin (Mel) signal acts on the pars tuberalis (PT), located at the interface of the hypothalamus and the pituitary, where it is involved in the seasonal regulation of prolactin secretion. The PT expresses Mel receptors and is believed to relay *via* a paracrine mechanism photoperiodic signals to pituitary lactotrophs.

Recent studies have shown that the clock genes *per1* and *cry1* are expressed in a rhythmical fashion in the PT, with *per1* tracking light onset/melatonin decline, and *cry1* tracking onset of melatonin secretion, and changes in *per/cry* phasing may provide a mechanism for decoding the melatonin signal in photoperiodism.

To investigate transcriptional pathways involved in this mechanism, we undertook a microarray experiment using PT tRNA from sheep placed in either long (LP) or short photoperiods (SP) and culled at three different time points: ZT4, 12 and 20, hybridized against a normalized 15K brain bovine cDNA library.

Our results show 2 distinct outlier clusters of only 4-5 clones in each, differently expressed in LP compared to SP at ZT12. The first one represents clones that are down regulated, and predictably *cry1* is found in this group. We characterized *neuroD1* and *pbef* as belonging to this cluster and investigated their mRNA expression in PT from sheep placed in SP. *In situ* hybridization results show an upregulation of both mRNA at ZT11 compared to ZT4. Interestingly, both genes are known to be involved in insulin regulation, pancreatic development, suggesting that these pathways may have been co-opted for photoperiodic time measurement in the PT.

16:30 - 17:00 Session 2: Physiology

Dr Tim Boswell – Newcastle University, UK

Development and use of a chicken neuroendocrine cDNA microarray in reproductive physiology

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We have constructed a chicken cDNA microarray enriched for neuroendocrine system genes as a tool for studying basic mechanisms underpinning reproductive physiology. The array was made in the ARK-Genomics laboratory at Roslin Institute and consists of 4800 cDNA fragments (200-500 bp). These clones comprise neuroendocrine-related genes selected from the BBSRC ChickEST collection (www.chick.umist.ac.uk); PCR products of chicken and quail neuroendocrine genes cloned in our laboratory; and anonymous cDNA clones derived from subtractive libraries made from whole hypothalamus, with separate reciprocal subtractions made between birds exposed to short or long days, and between laying hens and those in the early stages of incubation.

We have applied the chicken neuroendocrine array to isolate and characterize genes in the hypothalamus of the brain that are involved in mediating the stimulatory effects of long days on the reproductive system and, in a separate study, to identify differences in gene expression between laying hens and those in the early stages of incubation. We have identified a number of differentially expressed genes from these altered reproductive states and have been able to confirm differential expression in subsequent follow-up studies using quantitative-competitive PCR. Collectively, our results demonstrate the utility of the chicken neuroendocrine array as a tool for studying molecular physiology in the chicken.

17:00 - 17:30 Session 2: Physiology

Dr Ian Dunn – Roslin Institute, UK

Hypothesis generation from chicken shell gland micro-array experiments

Ian Dunn, Dave Waddington Roslin Institute, Maureen Bain Glasgow University Veterinary School

The oviduct of domestic hen grows and regresses with the activity of the reproductive system. The change in mass stimulated by ovarian steroids is around 30 fold and is accompanied by a prodigious capacity to secrete the components of the egg, including the shell. The eggshell forms in the shell gland region of the hen's oviduct over about 20 hours and is a highly ordered bio-ceramic of fused calcite (CaCO₃) crystal pillars formed on a protein skeleton with distinct layers and regular pores which allows gas exchange for the developing embryo. If the formation of the eggshell is compromised then an egg can suffer damage during handling and allow food spoilage organisms to enter the egg. Eggshell quality is therefore a priority for producers, retailers and consumers.

We aimed to increase our understanding of the molecular mechanisms by which the shell gland forms eggshells using data derived from microarray experiments. We used quantile normalisation of microarrays of juvenile (12 weeks of age) and sexually mature (24 weeks of age) hens to utilise data gathered in experiments comparing genetic lines of white leghorns. This produced a list of genes with expressions levels that were up or down regulated in the mature shell gland compared to the juvenile and which did not overlap. In a list of 300 potentially differentially expressed genes FDRs were in smaller than $1/1 \times 10^{-10}$. Real time PCR confirmed the differential expression of selected genes. As expected a number of genes which encoded for proteins that are known to be part of the eggshell such as ovocalyxin-32, ovocleidin-116, ovocalyxin-22, ovocalyxin-36 or part of the CaCO₃ secretory pathway such as carbonic anhydrase II and calbindin were present in the list. There were, however, many other genes we would not have predicted; amongst these were osteoprotegerin and bone morphogenic protein 3. We have established hypothesis on how these genes may maintain the shell gland in an active state and may act to control calcium homeostasis. Osteoprotegerin is a decoy receptor which modulates the differentiation of bone reabsorbing cells, the osteoclasts. We have hypothesised that the shell gland is a source of circulating osteoprotegrin which has an endocrine effect on mobilisation of calcium from bone by osteoclasts. Osteoprotegerin also prevents apoptosis by its ability to bind TRAIL and so we have further hypothesised that osteoprotegerin prevents apoptosis in the shell gland mediated by bone morphogenic proteins when the reproductive system is active.

In conclusion we have been able to derive new hypothesis on the function of the shell gland which when tested will lead to a greater understanding of the its function and how a quality shell is formed.

17:30 - 18:00 Session 2: Physiology

Dr Paul Sear – Cardiff University, UK

Identification of genes and gene pathways involved in the freshwater to seawater adaptation of the Atlantic salmon (*Salmo salar*).

P.J. Seear¹, S.A.M. Martin², J.B. Taggart³, J.E. Bron³, F.D. Murray⁴, R. Talbot⁴, D.F. Houlihan², C.J. Secombes², G.E. Sweeney¹, A.L. Archibald⁴, D.W. Burt⁴, B. Hoyheim⁵ and A.J. Teale³.

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⁴ARK-Genomics, Roslin Institute, UK;

⁵Norwegian School of Veterinary Science, Oslo, Norway

The UK Atlantic salmon aquaculture industry must meet a number of challenges if it is to remain sustainable and profitable. Constraints on commercial production include a.) the supply of dioxin-free highly unsaturated oils for the salmon diet, b.) protein growth efficiency, c.) infectious disease, and d.) a long and complex lifecycle. The project TRAITs (TRanscriptome Analysis of Important Traits in Salmon), was initiated to tackle these constraints (see <http://www.abdn.ac.uk/sfirc/salmon/>). This project is a partnership involving three UK universities (Aberdeen, Cardiff and Stirling) and the Norwegian Salmon Genome Project, supported by ARK-Genomics and working in collaboration with, Operon and Marine Harvest. The goal of TRAITs is to develop an oligonucleotide array for probing key elements of the transcriptome involved in polyunsaturated fatty acid metabolism, protein catabolism, responses to bacterial and viral challenge, and freshwater to seawater adaptation (smoltification).

A preliminary deliverable of this project, a 17K gene Atlantic salmon cDNA microarray was constructed using ESTs from the EU funded Salgene project and the Norwegian Salmon Genome Project, along with subtractive library clones generated by Aberdeen, Cardiff and Stirling. At Cardiff University we used the cDNA microarray to identify genes differentially expressed during smoltification. Cy3 and Cy5 labelled RNA from pituitary, brain, gill and kidney tissues were hybridised to the microarray in sextuplicate and with dye flips. Following data extraction, normalisation and statistical analysis, lists of more than two fold up and down regulated genes were obtained. Approximately 60-70 genes were found to be up regulated in kidney and gill and between 15-30 genes up regulated in pituitary and brain. Currently we are verifying differential expression of some of these genes with quantitative real-time PCR. The microarray and associated resources are available through ARK-Genomics (<http://www.ark-genomics.org>).

Thursday September 28th09:00 - 9:30 Session 3: Disease and Immunology *continued*

Dr Fuquan Zhang – Institute of Animal Health, UK

Macrophage transcriptional responses following in vitro infection with African swine fever virus (ASFV): a microarray-based studyFuquan Zhang,¹ Paul Hopwood,² Charles C. Abrams,¹ Alison Downing,³ Frazer Murray,³ Richard Talbot,³ Alan Archibald,³ Stewart Lowden,² and Linda K. Dixon^{1*}

Institute for Animal Health Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF, United Kingdom¹; ARK-Genomics Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom²; and Royal Dick Veterinary College, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, United Kingdom³

We have constructed a porcine cDNA microarray containing 2880 cDNA reporters, and used this array to investigate the response of porcine alveolar macrophages to ASFV infection by a high-virulence ASFV isolate, Malawi Lil20/1, or a low-virulence isolate, OUR/T88/3, at two time points, 4 h and 16 h post-infection. Differential regulated genes compared with mock infection were identified by a variety of algorithms, and clustered according to their expression profiles. Some of them were validated by real-time qPCR and protein expression.

This study is a useful first step in screening a wide range of host genes for potential ASFV-induced changes in RNA levels and provides a preliminary identification of some of the host genes that may be differentially regulated. Data from the microarray experiments provide a foundation for further studies, at the functional level, into the molecular basis of ASFV pathogenesis and virus mechanisms of immune evasion.

09:30 - 10:00 Session 3: Disease and Immunology *continued*

Dr Tahar Ait-Ali – Roslin Institute, UK

Dissecting PRRSV innate immune response in swine

T. Ait-Ali^{1*}, A. Wilson¹, S. Ganeshprasad¹, D. Wescott², M. Waterfall¹, M. Clapperton¹, M. Mellencamp³, D. Waddington¹, D. Mouzaki¹, T.W. Drew², S. Bishop¹, and A. Archibald¹.

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It is increasingly recognized that genetic components involved in host susceptibility represent an important step forward for the development of a disease control programs for PRRS. The present study was undertaken to evaluate the innate immune response of the alveolar macrophage of commercial pigs to PRRSV infection. Here we report a comparison of the *in vitro* replication of PRRSV and the genome wide transcriptional host response in alveolar macrophages derived from five genetic lines including Large white, Pietrain, Landrace and two synthetic lines selected for dam line robustness and efficient lean growth. First, we established that the bronchoalveolar lung fluid preparations of each line were essentially composed of macrophages/monocytes cells and derived from healthy piglets. We next showed that, *in vitro*, PRRSV kinetics of replication and growth in the Landrace alveolar macrophage animals were significantly and reproducibly reduced or delayed when compared with the other four lines. As no secondary pathogens were detected, this crucial information indicated the possibility that either intrinsic or apparent genetic factors are likely to be responsible for the *in vitro* attenuation of PRRSV replication. While no change in sialoadhesin PRRSV co-receptor accumulation and localization were detected genome-wide transcript analysis using the Affymetrix platform (24,123 genes monitored) was investigated. Groups of transcripts associated with this PRRSV susceptibility and reduced-susceptibility were identified and mapped, *in silico*, on the porcine genome. This effort will be discussed in conjunction with kinetics of cytokine productions. Taken together, this work reveals for the first time the transcriptional map of the innate immune response in alveolar macrophage of two breeds showing different level of susceptibility to PRRSV infection and indicates signalling pathways likely to operate during PRRSV infection.

10:00 - 10:30 Session 3: Disease and Immunology *continued*

Dr Sam Martin - University of Aberdeen, UK

Transcriptional analysis following recombinant interferon stimulation on an Atlantic salmon (*Salmo salar*) cell line SHK-1

S.A.M. Martin, J. Zou, D.F. Houlihan & C.J. Secombes

Interferons (IFN) are cytokines that have proinflammatory, antiviral and immunomodulatory effects, and as such play a central role during a host response to a pathogen. The IFN family contains both type I and type II molecules. Whilst there are a number of class I IFNs, there is only one class II cytokine. Recently both type I and type II IFN genes have been cloned in salmonid fish and recombinant proteins produced indicating they possess IFN activity. We have stimulated an Atlantic salmon cell line SHK-1 with both recombinant type I and type II salmonid IFNs and analysed the transcriptional response by microarray analysis. Cells were stimulated with recombinant cytokine for 6 or 24 h or unstimulated as control. RNA was used to hybridize to an Atlantic salmon microarray (salmon TRAILS array). The microarray contained 16,950 Atlantic salmon ESTs plus various positive and negative controls, all features were printed in duplicate. Differentially expressed genes were found to be stimulated by only one interferon type, however a number were shown to be affected by both interferons indicating there may be co regulation of the IFN response in fish. Several genes found to be altered in expression were used in additional real time PCR analysis as further confirmation of the results obtained by microarray. The TRAILS microarray was constructed by ARK Genomics and funded by BBSRC grants EGA17675 (Salmon TRAILS) and BB/C506021/1.

10:30 - 10:40 Dr Heike Kofler - Operon Biotechnologies GmbH

New Platforms and Systems for DNA Microarrays: 70mer Oligonucleotide Probes Optimised for Animal Expression Analysis

Heike Kofler, Beate Saal, Kirsten Wellesen, Björn Henze, Denja Drutschmann and Peter Schuessler

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DNA microarrays are commonly used in expression profiling and genetic analysis experiments. Today still many DNA arrays are based on short oligonucleotides or cDNAs. These can provide either high specificity or high sensitivity respectively, but cannot combine these qualities. Operon has designed probes of different lengths to various positions in the Open Reading Frames (ORFs) and the results clearly show that 70mers offer the optimal combination of specificity and sensitivity.

Using the Ensembl database <http://www.ensembl.org> or other databases that display genome and transcript information allows the design of common, partial common and individual transcript 70mers. This design strategy is suitable for differentiating alternative splice variants and is maximizing the number of represented transcripts. Whenever possible, oligos were designed to be fully contained in one exon (exon oligos) avoiding the location of oligos across exon borders. Therefore exon oligos are also applicable for CGH (comparative genome hybridizations) experiments.

In collaboration with leading researchers in the field of animal sciences, Operon has designed Array-Ready Oligo Sets™ consisting of optimized 70mers for expression analysis on genome and transcript level for a number of genomes, including Bovine, Pig, Human, Mouse, Rat, Listeria, Sinorhizobium, Rice, Maize and Tomato.

To help users deal with microarray experiment data, we have linked our Oligo Microarray Database (<http://www.operon.com/arrays/omad.php>) to the corresponding public databases. Therefore our users can always access the latest information for target genes and transcripts.

Experiments and user data demonstrate the specificity, sensitivity and reproducibility of results obtained by applying 70mers for expression profiling arrays and CGH arrays.

11:00 - 11:30 Session 4: Physiology *continued*

Prof Claire Wathes – Royal Veterinary College, UK

Effect of energy balance status on gene expression patterns in the *post partum* dairy cow.

D.C. Wathes¹, M. Fenwick¹, Z. Cheng¹, S.D. McCarthy^{2,3}, D.G. Morris², M.G. Diskin², D. Kenny³, J. Patton⁴, J. Murphy⁴ and R. Fitzpatrick²

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In early lactation dairy cows cannot consume sufficient nutrients to support milk production. They therefore enter a period of negative energy balance (NEB) during which body tissues are mobilised. NEB is associated with extensive biochemical and morphological modifications in the liver. These are necessary to maintain glucose homeostasis but can have adverse impacts elsewhere in the body, notably reducing fertility. This study utilized a model of NEB in the dairy cow based on variations in nutrient supply and milking frequency to produce 2 groups of varying EB status. Monitoring of blood metabolites and circulating IGF-I in early lactation confirmed that a differential EB status had been achieved. Tissues harvested at 2 weeks *post partum* from cows in mild MNEB and severe SNEB were screened using bovine 23K microarrays (Affymetrix) with RNA isolated from liver and uterine endometrium. Normalised array data was analysed using the local pooled error (LPE) test with the false discovery rate controlled to 5%. In the liver, 524 genes were significantly differentially expressed. Validation studies using qPCR confirmed that key IGF related genes expressed in liver were either down- (GH-R, IGFBP-3 and the acid labile subunit ALS) or up-regulated (IGFBP-2). These changes would be predicted to alter the half life and bioavailability of circulating IGF and may therefore affect the functionality of reproductive tissues. Within endometrium, cows in SNEB showed histological evidence for higher levels of inflammation. From the array analysis, 497 genes were differentially expressed, particularly in categories relating to tissue turnover and immune response. These results suggest that animals in SNEB have reduced capacity to clear uterine infections that are prevalent after calving in dairy cows. This may contribute to the observed reduction in fertility in such animals.

11:30 - 12:00 Session 4: Physiology *continued*

Dr Tony Jackson – Cambridge University, UK

Life and death without clathrin in chicken DT40 lymphocytes

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We have exploited the high rate of homologous recombination shown by the chicken pre-B cell line DT40 to delete both endogenous copies of the clathrin heavy-chain gene and replace them with clathrin under the control of a tetracycline-regulatable promoter (Tet-Off). The originally derived cell-line DKO-S undergoes apoptosis when clathrin expression is repressed. The partial inhibition of transferrin uptake is responsible for the lethal consequence of clathrin depletion. We have also described a cell line DKO-R, derived from DKO-S cells, that is much less sensitive to clathrin depletion. To investigate the reason for the different sensitivities of the two cell-lines to clathrin depletion, gene expression profiles were compared between DKO-S and DKO-R cells using microarray analysis. This identified an anti-apoptotic signaling pathway based on the chemokine receptor CXCR4 and its ligand SDF1 as being up-regulated in DKO-R cells. The work clarifies a puzzling feature of the behaviour of clathrin-depleted DT40 cells, places clathrin function within a wider physiological context and reveals a striking example of how pro and anti-apoptotic signaling pathways combine to regulate cell survival.

13:00 - 15:00 Session 5: Parallel Workshop Sessions

Poster Session

GO annotation & Reactome [*run back-to-back*]

Arkdb & Microarray normalisation (2-dye) [*run back-to-back*]

15:30 - 16:30 Session 6: Genomics - outlook and visions

Dr Steve Picton – Affymetrix

Targeted Genotyping

Dr Evelyn Camon, Dr Jennifer Clark – European Bioinformatics Institute (EBI)

Overview of GO tools

Gene Ontology Annotation (GOA) database

(E Camon and E Dimmer)

The Gene Ontology (GO) is a well-established, structured vocabulary that has been successfully used for 8 years in the annotation of proteins. GO terms, created in consultation with the biology community, are used to replace the multiple nomenclatures used by scientific databases that can hamper data integration. Currently GO consists of more than 20,500 terms distributed over three ontologies that describe the molecular function, process and location of action of a protein in a generic cell. The Gene Ontology Annotation (GOA) database at the EBI (<http://www.ebi.ac.uk/GOA>) aims to provide high-quality manual and electronic GO annotations to proteins within the UniProt Knowledgebase. By annotating all 'known' proteins with GO terms and transferring this knowledge to highly similar 'unknown' proteins, GOA offers a valuable contribution to the understanding of all proteomes. GOA provides annotated entries for over 110,000 species and is the largest and most comprehensive open-source contributor of annotations to the GO Consortium annotation effort. In addition, by integrating GO annotations from model organism groups (FlyBase, GeneDB, HGNC, MGI, RGD, SGD, TAIR, Gramene, TIGR, ZFIN, AgBase, WormBase, Reactome, IntAct, LIFEdb, DictyBase and Roslin Institute), GOA ensures the dataset remains a key reference. For several years GOA has been the only GO Consortium member responsible for the submission of human GO annotation file. More recently we have become responsible for the release of the bovine and chicken GO annotation datasets in collaboration with UCL, AgBase and the Roslin Institute. GO annotations to a number of other farm animal species can be downloaded from our UniProt-GOA file. All GOA datasets can be queried through a user-friendly web interface via our QuickGO browser (<http://www.ebi.ac.uk/ego>) or downloaded in a parsable format via the EBI (<ftp://ftp.ebi.ac.uk/pub/databases/GO/goa>) and GO FTP sites. GOA welcomes feedback from the farm animal research community and can assist new groups with annotation tools and mentoring. Researchers wishing to query or contribute to the GOA project are encouraged to email: goa@ebi.ac.uk.

Are your gene products of interest annotated to the GO?

J. I. Clark and the Gene Ontology Consortium

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The GO Consortium conducts GO Annotation Camps to introduce new groups to the GO system. We also pair new groups learning to use GO terms to annotate a genome with more experienced 'mentor' groups. To further aid annotating groups, the GO project provides links to free, open source annotation software that can be used by any group, and mailing lists where advice can be sought on any GO related matter. For any questions on the GO project, or for assistance using the system to annotate your genome or gene products of interest, please contact the GO mailing list on gohelp@geneontology.org. (poster ID#13)