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### A1–COMPARATIVE GENOMICS AND PROTEOMICS AND IDENTIFICATION OF NEW MOLECULAR TARGETS FOR DRUG ACTION

Organised by Dr. John Parrington (University of Oxford) and Dr. Kevin Coward (University of Oxford)

#### A1.1 Comparative genomics of vertebrates

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Our genome differs from those of other vertebrates in many respects: we have gained ‘new genes’ by duplication and lost others by pseudogenisation; sequences have diverged by drift and adaptation; gene expression profiles have changed; and our chromosomes have suffered fissions, fusions and inversions. By drawing on the newly available genome sequences of rodents, chicken, chimpanzee and dog, I will explore the sluggish or rapid rates of these processes. I will give examples of how our genome differs from these other vertebrates and how rates of mutation contrast with those of selection. I also show how evolution has impacted on Mendelian ‘disease genes’ differently from other genes.

Keywords: Disease genes, Adaptive evolution, Chromosomal rearrangement, Selection, Mutation

#### A1.2 The sea urchin genome: resources of a basal deuterostome for comparative genomics

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Together with the hemichordates, sea urchins represent basal groups of nonchordate invertebrate deuterostomes that occupy a key position in bilaterian evolution. The sea urchin system has a long and extremely successful history as a model organism and continues to be the animal model of choice for many embryologists because of the easy and unlimited availability of masses of eggs and sperm. For

example, mechanisms of general biological importance such as egg activation, fertilization, calcium signaling, cell cycle, and exocytosis have been elucidated in sea urchins as well as the identification of molecular mechanisms that underlie cell movements during gastrulation or axis-specification. The power of the system has now been extremely strengthened through the deciphering of its transcriptome and its genome. I will summarize the status of several sequencing projects and give an overview on genomic tools, methods and databases that are available.

#### A1.3 Nicotinic acetylcholine receptors as drug/chemical targets: contributions from comparative genomics, forward and reverse genetics

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Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels. They play a key role in fast cholinergic synaptic transmission in vertebrates and invertebrates. In man, nAChRs are targets for drugs being developed with the aim of ameliorating the symptoms of Alzheimer’s disease and others designed to relieve neuropathic pain. Invertebrate nAChRs are targets for drugs/chemicals controlling human and animal parasites as well as crop pests. Comparative genomics and physiology have shown how alternative splicing, RNA editing and multiple isoforms contribute to generating molecular and functional diversity in nAChR subunit families (1,2). Strikingly different actions of amyloid peptides, which play a central role in Alzheimer’s disease, have been observed in studies on recombinant human brain nAChR subtypes. Chemistry-to-gene screens in *C. elegans* enable the identification from a very large receptor family of a small number of nAChR

subunits which are targeted by an antiparasitic drug (3). A combination of subunit gene expression profiles and patterns, together with RNA interference and physiology, have helped establish roles for particular nAChR subunits in cholinergic neurons of *D. melanogaster*. High-throughput RNAi screens may offer new approaches to drug target discovery and validation.

(1) Sattelle et al. (2005) *Bioessays* 27, 366–376.

(2) Jones and Sattelle (2004) *Bioessays* 26, 39–49.

(3) Jones et al. (2005) *Nature Rev Drug Discov* 4, 321–330.

#### A1.4

##### Application of neurogenomics and neuroproteomics for CNS drug discovery

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Withdrawn

#### A1.5

##### Discovery of novel voltage-gated sodium channel inhibitors—a gene family-based approach

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Voltage-gated sodium ( $\text{Na}_v$ ) channel inhibitors are an important class of drugs that are used to treat a number of CNS indications including pain, local anaesthesia, epilepsy and bipolar disorder. These drugs all have their origins in traditional “empirical” pharmacology, and it was only some time after discovery that they were found to inhibit  $\text{Na}_v$  channels. The basis for therapeutic selectivity of these drugs within different disease indications is currently unknown. However, the subsequent discovery of a multi-gene family of  $\text{Na}_v$  channels suggests a possible mechanism and has opened the way for more targeted approaches to finding improved therapeutic inhibitors. This presentation will describe our ongoing approaches to systematically clone, express and characterise the entire family of  $\text{Na}_v$  subtypes in order to better understand their properties and define their individual physiological and pathophysiological roles. As well as providing specific disease validation for individual subtypes, this also provides a panel of reagents for comprehensively exploring the efficacy, selectivity and potency relationships of existing  $\text{Na}_v$ -blocking drugs. In this way, a gene family-based approach to  $\text{Na}_v$  channels has enabled a “drug-to-target” approach, reversing the more usual “gene-to-target-to-drug” paradigm. Together with recent advances in assay technology, gene family-based approaches are increasing the tractability of these targets and are re-invigorating  $\text{Na}_v$  drug discovery within the pharmaceutical industry.

Keywords: Sodium channel, Gene family, Subtype, Inhibitor, Drug discovery

#### A1.6

##### Pharmacogenetics in Pharmaceutical Development

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Pharmacogenetics studies inherited variability in individuals and populations in response to the safety and efficacy of medicines. Early studies focused on the enzymes and genes involved in the metabolism and excretion of drugs and led to the identification of the molecular understanding of poor metabolism in some individuals taking drugs that were metabolised by enzymes such as cytochrome P450, CYPD6. The value of understanding variability in these and similar classes of genes has led to the development of new drugs that use different mechanisms for metabolism avoiding some of the commoner issues of inherited variation. In the past 10 years studies have started to understand other common safety issues seen in drug development for example hypersensitivity reactions and hyperbilirubinaemia. How these results are having an impact on the development of new drugs and the regulatory environment will be discussed.

#### A1.7

##### The practical implications of comparative kinetoplastid genomics

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*Leishmania* spp., *Trypanosoma brucei* spp. and *Trypanosoma cruzi* are all major human pathogens, causing respectively the diseases leishmaniasis, sleeping sickness and Chagas disease. They all belong to the ancient eukaryotic group of Kinetoplastida and share many distinct morphological, biochemical and metabolic properties and yet have very different interactions with the host. Together, they cause disease and death in millions of humans, with a fifth of the world's population at risk. There are currently no vaccines and what few drugs are available are toxic, difficult to administer and suffer from problems of increasing drug resistance. The complete genomes of *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi* have been sequenced and recently analysed with respect to each other. As well as revealing new and fascinating insights into the biology of this unique group of organisms, it has also identified kinetoplastid specific genes, shared between the species. Many of these are known to be part of the core proteome, while others have no predicted function and are likely to encode proteins involved in host pathogenicity. Kinetoplastid specific genes and those that have been potentially laterally acquired from prokaryotes (and are therefore phylogenetically distant from humans) can be considered for further investigation as new targets for drugs, vaccines and diagnostic tool development.

#### A1.8

##### Genomic approaches to identifying new targets for anti-malarial drugs

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Plasmodium is the causative agent of malaria, one of the most prevalent and severe human infectious diseases. Anopheles mosquitoes inject sporozoites into the host, which rapidly migrate to the liver, invade hepatocytes and develop into merozoites that are released to the blood stream initiating the clinical phase of

infection. Because liver infection is the first obligatory step of infection, hepatocyte–Plasmodium interactions crucial for the establishment of infection constitute an ideal target for potential anti-malarial vaccines or preventive treatments.

Plasmodium sporozoites are able to enter any cell tested so far but only develop in a very restricted type of cell, such as hepatocytes and hepatoma cell lines. This strongly suggests a crucial role of the host cell in sustaining the growth and development of Plasmodium. The reason, however, why Plasmodium sporozoites are only able to develop in certain cell types remains unknown. We propose to determine the host cell molecules and mechanisms required for proper parasite development inside host cells.

Our laboratory has established recently an in vitro system to monitor the development of Green Fluorescent Protein (GFP) expressing sporozoites in hepatoma cell lines such as HepG2 and Hepa1-6 cells which allow the full development of *P. berghei* sporozoites. Using a microarray approach we have already determined a list of host cell genes which expression is altered by Plasmodium sporozoite development inside hepatoma cells. Now, we propose to determine which of these host cell molecules and mechanisms involved are required for proper parasite development inside hepatocytes. In order to identify them, we propose to apply a systematic RNA interference (RNAi) screen to this assay. Our preliminary results show the feasibility of such a screen. We expect to provide a significant contribution to the understanding of the mechanisms mediating Plasmodium–hepatocyte interactions, which might have important implications in the development of new strategies against malaria. Identifying the host proteins that either promote infection or help ward them off not only shed light on the basic mechanisms of malaria infection but also provide potential new targets for disease control.

### A1.9

#### Target validation: pick and mix

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There are a range of target validation technologies available to the pharmaceutical industry but true target validation only comes with the recognition that a medicine is safe and effective in the patient. So the idea that any platform technology generates ‘validated targets’ is appealing but only partially true. Target validation is at its most convincing if knowledge is integrated from different sources and within a recognisable scheme. The pharmaceutical industries target validation process has to take account of the ‘druggability’ of targets. Genomic analysis showed us how many of these ‘target class’ proteins there, reminds us that they are finite (around 3000 at present) and shows us that these targets (and pathways) are not always shared between species. Animal models of human diseases are not always the right way forward, or even possible. A ‘target validation’ checklist might typically involve orthologue, expression, knockout and genetic analysis in that order. We already have genome scale information on the first two. Transgenic mice and other model organisms such as *C. elegans*, *D. melanogaster*, and *D. rerio* provide additional information. RNAi technologies enable an evaluation of human gene function in a number of backgrounds. Each approach has a particular application—it has to be picked and mixed with other strands of evidence to build a convincing package of information. Human genetics is

beginning to contribute significant targets and, perhaps more importantly, it is drawing together clinical practice and molecular sciences. It is interesting that much of what we know of target validation has been learned from drugs developed without the influence of the genomic or genetic technologies available to us now. There is a premium on associating our best leads with their most likely therapeutic use (and, sadly, this is not always the one we first thought of). Our challenge is to make use of all the data at our disposal. In the long run the temptation to look at only a part of the data, only at the ‘druggable targets’ or at evidence consistent with familiar pathologies, should be avoided as genomic and genetic sciences generate information without reference to our prejudices. These points argue for more, better and wider gene annotation. This provides our best chance of generating hypotheses and so success as technologies now generate genome scale data.

Keywords: Drug discovery, Genetics, Genomics, Model organisms

### A1.10

#### NAADP and cADPR signalling in sea urchin eggs

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Cyclic adenosine diphosphate ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) are newly described intracellular messengers for calcium signalling. Originally discovered in the sea urchin egg, these molecules have now been shown to have widespread actions in many cell types, including many relevant to patho-physiological processes. Our working hypothesis is that NAADP is a most potent initiator of calcium signals, whereas cADPR amplifies calcium signals through the sensitization of calcium-induced calcium release through the modulation of ryanodine receptors. These molecules can work independently or in concert with inositol trisphosphate to determine specific calcium signalling patterns (and hence responses) in cells. To gain new insights into the molecular components of the cADPR and NAADP signaling pathways, we are utilizing an important new resource—a sea urchin egg and embryo cDNA database. The aim of this work is to (a) identify proteins involved in these novel calcium signalling pathways, (b) generate tools to dissect the molecular mechanisms underlying these pathways, (c) determine interactions between signalling components, (d) identify functional role of proteins in urchin eggs and during embryogenesis (e). We will describe progress in identifying components of these important signalling pathways and their functional roles.

### A1.11

#### Polymorphisms of the porcine *Bone Morphogenetic Protein Receptor 1 Beta (BMPR1B)* gene

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The Booroola phenotype (FecB) has been fully associated to a single amino acid substitution (Q249R) in the *Bone Morphogenetic Protein type 1 $\beta$  receptor* (*BMPR1B*). Homozygous FecB<sup>B</sup> ewes are hyperprolific as a consequence of the precocious development of a large number of small antral follicles that leads to an increased ovulation rate. In pigs, *BMPR1B* maps to chromosome 8 within the interval of a QTL affecting ovulation rate. We have sequenced the complete coding region of the porcine *BMPR1B* ovarian cDNA. Three silent mutations were identified within exon 10 of the gene: G/C<sub>804</sub>, C/T<sub>852</sub> and C/T<sub>960</sub>. An Iberian  $\times$  Meishan F<sub>2</sub> resource population, with records on reproductive traits, was genotyped for the 3 mutations. Segregation analysis revealed the existence of 4 different haplotypes within this population: A (G<sub>804</sub>–C<sub>852</sub>–C<sub>960</sub>), B (G<sub>804</sub>–C<sub>852</sub>–T<sub>960</sub>), C (C<sub>804</sub>–T<sub>852</sub>–T<sub>960</sub>) and D (C<sub>804</sub>–C<sub>852</sub>–T<sub>960</sub>). A representative sample of 82 pigs from different breeds was genotyped for estimating the allelic frequencies (14 Pietrain, 13 Large White, 14 Landrace, 13 Duroc, 10 Iberian and 18 Meishan). Haplotype A was predominant in the European breeds, with a frequency ranging from 0.5 in the Large White population to 0.85 in the Iberian pigs; whereas haplotype D was the most frequent in Meishan. Haplotype C only appeared in the Meishan breed suggesting a possible Asiatic origin of this haplotype. An association analysis between *BMPR1B* polymorphisms and sow reproductive traits will be performed in the Iberian  $\times$  Meishan F<sub>2</sub> population.

Keywords: *Bone Morphogenetic Protein Receptor 1 $\beta$* , pig, polymorphism

### A1.12 Phylogenetic relationships among diverse African and South American pig breeds

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Present day pig breeds descend from the wild boar (*Sus scrofa*) which was domesticated 9000 YBP in multiple locations across Eurasia. Two main mitochondrial Asian (A1, A2 and A3) and European (E1 and E2) lineages have been identified so far, with an estimate time of divergence of 58,000–900,000 YBP. The clustering of European standard breeds, such as Large White, with the Asian clade has been interpreted in the light of the extensive introgression with Chinese breeds that these European populations went through the 18–19th centuries. In the current work, we have analysed genetic variation of the mitochondrial cytochrome B gene and D-loop region of South American and African pig populations, which descend from the ones brought by the European colonizers several centuries ago. Peruvian creole ( $N=8$ ) and Pampa Rocha (Uruguay,  $N=6$ ) pig populations displayed exclusively European haplotypes, a feature which is consistent with the influence of the Iberian pigs brought by the Spanish conquerors. In the Nicaraguan creole breed ( $N=6$ ), we only found Asian haplotypes suggesting a Chinese

origin, as previously reported for the Cuino Mexican breed. Nigerian ( $N=10$ ) and Benin ( $N=3$ ) pigs showed European haplotypes, whereas the Mukota breed (Zimbabwe,  $N=4$ ) displayed Asian (A1=0.5, A3=0.25) and European (E1=0.25) haplotypes. Our results suggest the existence of a relationship between the frequency of Asian haplotypes in South American and African pig populations and the historical timepoints at which these breeds were formed.

Keywords: Domestic pig breeds, Mitochondrial DNA, Phylogenetic analysis

### A1.13 Polymorphism of the goat lipoprotein lipase gene

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Lipoprotein lipase (LPL) is a lipolytic enzyme involved in the metabolism of triacylglycerol-rich lipoprotein particles. It hydrolyzes the triacylglycerol cores of chylomicrons and low density lipoproteins, generating free fatty acids available for extrahepatic tissues. The activity of the milk LPL has been shown to differ amongst several goat breeds, suggesting the existence of a genetic polymorphism influencing the functional properties of this enzyme. Here we describe the isolation and molecular characterization of the cDNA sequence of the goat *LPL* gene. The goat *LPL* nucleotide sequence was 2548 bp long, encoding a protein of 478 amino acids. This sequence included part of the 5'UTR (294 bp) and 3'UTR (820 bp). The goat LPL protein shared a high amino acid identity (99%) with the ovine and bovine LPL orthologous sequences. Several structural motifs that are common to LPL and other functionally related enzymes were identified in the goat LPL sequence. These motifs included a PLAT domain, which might be involved in protein–protein and protein–lipid interactions. We have also observed a triglyceride lipase domain containing a serine residue that, together with two other histidine and aspartate residues, participates in a charge relay system.

Keywords: Lipoprotein lipase gene, Goat, Milk fatty acids

### A1.14 Identification of a polymorphic LINE insertion in the 6th intron of the porcine malate dehydrogenase 1 (*MDH1*) gene

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Pig malate dehydrogenase 1 (*MDH1*) catalyses the reduction of oxalacetate to malate producing NADPH. Moreover, the MDH1 enzyme is integrated in the tricarboxylate shuttle, which transports acetyl-CoA molecules from the mitochondria to the cytosol. Both NADPH and acetyl-coA are required for fatty acid biosynthesis. The pig *MDH1* locus has been mapped to chromosome 3 (Wintero et al., 1998) and the corresponding cDNA has been partially characterized

(Trejo et al., 1996). We have sequenced the *MDHI* cDNA in 12 pigs belonging to five breeds (Piétrain, Large White, Landrace, Vietnamese and Iberian) with the aim of characterising the polymorphism of this gene. One silent C/T polymorphism at exon 7 was found by aligning these sequences. In addition, sequencing of a genomic fragment including exon 6, intron 6 and exon 7 allowed to identify a truncated 0.7 kb LINE L1Ss insertion at intron 6. This LINE insertion was polymorphic in the five analysed breeds, with frequencies ranging from 0.10 to 0.54. The amplification of the exon 6–7 region also yielded a 1.7 kb fragment sharing 84% nucleotide identity with the pig *MDHI* gene and containing four tandem SINE PRE-1 insertions. This later finding suggests the existence of a non-processed *MDHI* $\psi$  pseudogene in the pig genome.

Keywords: Pig, LINE, *MDHI* gene

Trejo et al. (1996) *Gene* 172, 303–308.

Wintero et al. (1998) *Mammalian Genome* 9, 366–372.

### A1.15

#### Nucleotide sequence and polymorphism of the pig acetyl-coenzyme A carboxylase alpha gene

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Acetyl-Coenzyme A carboxylase  $\alpha$  (ACACA) is a cytosolic enzyme essential for the biosynthesis of long-chain fatty acids. In human, the *ACACA* gene is 330 kb long whereas the corresponding mRNA has a size of 10 kb approximately. The *ACACA* gene has 64 exons, including 7 alternatively spliced minor exons (1A, 1B, 1C, 3, 5A', 5A and 5B). The transcription of the *ACACA* gene is regulated by three alternative promoters PI, PII and PIII which are located upstream of exons 1, 2 and 5A, respectively (Mao et al., 2003). In pig, the *ACACA* gene has been mapped to chromosome 12 (Calvo et al., 2000). In the current work, we have sequenced 4.3 kb of the pig *ACACA* cDNA (exons 3–29 and 37–45), a region which encompasses 66% of the coding region. The Blastn analysis of this sequence yielded a 92–93% nucleotide identity with its bovine and ovine orthologous sequences. Moreover, the amino acid translation of this sequence has allowed to identify three functional biotin carboxylase, biotinyl lipoyl and carboxyl transferase domains. Sequencing of the remaining 3 kb is currently underway. The main purpose of this work is to identify polymorphisms in the *ACACA* gene that might be used to perform an association analysis with fatness traits, fatty acid composition and cholesterol metabolism in a Duroc population.

Keywords: Pig, Acetyl-Coenzyme A carboxylase  $\alpha$

Calvo et al. (2000). *Cytogenet. Cell. Genet.* 90, 238–239.

Mao et al. (2003). *Proc. Natl. Acad. Sci. USA.* 100, 7515–7520.

### A1.16

#### Polymorphisms in the goat $\beta$ -lactoglobulin gene

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$\beta$ -Lactoglobulin ( $\beta$ LG) is the major whey protein in the milk of ruminants but it is not present in the milk of humans, rodents and lagomorphs. Different genetic variants affecting the  $\beta$ LG protein have been characterized in bovine and ovine, but not in caprine species. We amplified and sequenced the proximal promoter and the first six exons, containing the entire coding region, of the  $\beta$ LG gene in 11 goat breeds (Alpine, Saanen, Malagueña, Murciano-Granadina, Tinerfeña, Majorera, Palmera, Teramana, Girgentana, Cashemere and Sahelian) to identify genetic variants. Fifteen polymorphisms have been detected, nine in the promoter region and six in the exons. All polymorphisms were single nucleotide substitutions with the exception of one deletion/insertion in the promoter region. Polymorphisms in the coding region did not produce any amino acid change. In addition, pyrosequencing technology was used to genotype four polymorphisms in the promoter region (–134, –118, –64 and –60; relative to goat  $\beta$ LG gene sequence (1)) in 200 goats belonging to the 11 breeds. Polymorphisms –118 and –64 affected an AP-2 consensus binding site. The –64 (A/G) polymorphism was only found in Italian breeds with a higher frequency for the A allele in Girgentana goats, being a good candidate to explain differences in  $\beta$ LG content observed in animals of this breed. Polymorphisms –134 (T/G) and –60 (T/C) were widely distributed among all the analysed breeds. Finally, the –118 (T/C) was only found at low frequencies in the Alpine, Saanen and Malagueña breeds.

Keywords: Pyrosequencing, Haplotype, Whey protein, Caprine, Allelic frequencies

1 Folch J.M. et al. (1994). *J Dairy Sci* 77, 3493–7.

### A1.17

#### Gene expression dynamics during regeneration in a brittlestar—the Wnt pathway—I: Gene expression profiling

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Adult echinoderms are able to regenerate lost body parts by a poorly understood mechanism. Since regeneration involves cell lineage regulation and proliferation, it recalls embryonic development, but unlikely recapitulates development exactly, because it occurs in a different environment. During development, Wnt proteins induce changes in gene expression, cell migration and cell adhesion. To understand the role of the Wnt gene family in adult regeneration, we identified various Wnt paralogues in the brittle star *Amphiura filiformis* and characterized their expression.

Conserved Wnt sequence regions were located by multialignments and used to design degenerate Wnt primers. After PCR from limb derived cDNA followed by cloning, and sequencing, Wnt paralogues were characterized by phylogenetic and comparative approaches. For in situ hybridizations, Wnt clones were miniprepred and the vectors linearized before transcription into single stranded RNA digoxigenin labeled probes. Taqman primers and probes were also designed from the sequences, for real-time PCR studies. A number of *A. filiformis* Wnt sequences (AfWnt1, AfWnt2, AfWnt3, AfWnt5, AfWnt7, AfWnt8, and AfWnt10) are presented and characterized for quantitative gene expression profiling. The dig-labelled probes

also enabled localization of some Wnt paralogues in limb tissue by in situ hybridization (detailed in Dupont et al.; A5 session). Our genomic studies will clarify the relative role of Wnt paralogues in regeneration. Comparison to regeneration in planarians (invertebrates) or tail regeneration in salamanders (vertebrates), where Wnt genes are implicated, will also be possible. Such analyses can illustrate the versatility of the use of Wnt signaling in developmental and regeneration processes. Keywords: Regeneration, Wnt, Expression profiling, In situ hybridization

### A1.18 Generation and sequence characterization of a cDNA library from rabbit oviduct

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The oviduct is the reproductive organ where fertilization and early embryo development occurs. The analysis of gene profile expression in the oviduct is a strategy that can help further understanding of some of the molecular mechanisms involved in this early phase of gestation. We have generated a non-normalized cDNA library from rabbit oviduct samples taken at 62 h after-mating, the timepoint at which rabbit embryos are mainly in the morula stage and situated in the oviduct. The titre of the library obtained was  $6.8 \times 10^6$  cfu/ml and the rate of recombination was 96%. A total of 215 clones have been randomly isolated and single-pass sequenced from the 5' ends. Only those clones with an insert size >150 bp have been analyzed for homology to known genes using BLASTN. Although most of the cDNAs are not full-length, they are long enough to be characterized. Among the 176 ESTs analyzed, 90 matched with known and characterized mammalian coding sequences, 60 matched with sequences whose genes have not been annotated yet and 26 represented transcripts with no significant match to any sequence in the GenBank database. The ESTs analysis of this cDNA library provides functional data on genes that may be implicated in early embryo development, it is also a source of sequences of genes already known in other species but unknown in rabbit, and finally, it can also be a source of candidate genes for reproductive traits. Keywords: Rabbit, Oviduct, cDNA library, ESTs

### A1.19 SlimeBase: the transcriptome of *Aplysia californica*

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*Aplysia californica* is a marine opisthobranch mollusc used as a model species in neurobiology for cellular analyses of learning and behavior. The comparatively small number and large size of the

neurons has facilitated these studies. Consisting of 17 haploid chromosomes, the *Aplysia* genome is  $1.8 \times 10^9$  base pairs. The mollusca comprise the second largest animal phylum yet lack detailed genetic and genomic studies. While sequencing efforts on over 30 bilaterian species in the deuterostomes and ecdysozoa have been undertaken, no member of the lophotrochozoa has had its genome sequenced. Thus developmental and comparative evolutionary biology as well as biomedical research will benefit from genomic studies of *Aplysia*.

We have constructed a series of unidirectional cDNA libraries from different life stages of *Aplysia*. These include EGGS, VELI (veliger), JMAC (metamorphic), JUVE (juvenile) and ACAN (adult neural). Individual clones were randomly picked and high-throughput, single-pass sequence analysis was performed. The resulting expressed sequence tags (ESTs) were trimmed for quality, clustered, annotated and deposited into the public database, "SlimeBase", which is based on "FunnyBase" (Paschall et al., 2004). The cDNA clones will be used for printing microarrays for analysis of gene expression in a tissue- or stage-specific manner. The addition of new *Aplysia* ESTs to public databases will contribute to our understanding of evolutionary and developmental biology, and to the unravelling of the cellular physiology of memory.

Keywords: *Aplysia*, cDNA libraries, ESTs, Genomics

### A1.20 Characterization, polymorphism analysis and physical mapping of the porcine *CDS1* gene

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CDP-diacylglycerol synthase 1 (*CDS1*) catalyzes the conversion of phosphatidic acid to CDP-diacylglycerol, an important precursor for the synthesis of phosphatidylinositol, phosphatidylglycerol and cardiolipin. We amplified and sequenced the pig *CDS1* mRNA and a 411 bp fragment of the proximal promoter region. The 2053 bp cDNA contained 537 bp of 5'-UTR, 1923 bp of coding sequence and 133 bp of 3'-UTR. Pig *CDS1* mRNA codes for a protein of 461 amino acids with 96%, 95% and 94% identity to human, mouse and rat proteins, respectively. The genomic structure is very similar to that of the human, rat and mouse genes with respect to size and organization of the 13 exons. In addition, we sequenced 1248 pb of the *CDS1* mRNA from animals of five pig breeds (Iberian, Landrace, Large White, Piétrián and Meishan) to identify genetic variants. The comparative sequence analysis revealed three polymorphic positions in exons 10 and 11: A/C<sub>1006</sub>, C/T<sub>1037</sub>, G/T<sub>1038</sub> (positions are indicated respect the start codon). Two of them cause amino acid change (Pro/Trh and Ala/Val, respectively). Expression of this gene was analysed by real-time PCR in stomach, liver, muscle, testis, ovary and fat, and the highest *CDS1* expression was found in testis. Finally, the *CDS1* gene was physically mapped to porcine chromosome 8 (SSC8), at 26 centiRay (cR) of the microsatellite marker SW1980 (LOD=12.51) using the INRA-University of Minnesota porcine radiation hybrid panel (ImpRH).

Keywords: Pig, Sequencing, Expression, Mapping

### A1.21 TIMP-1 as candidate gene for embryo survival in rabbits

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In rabbit (*Oryctolagus cuniculus*) production, litter size is the most common criterion used to select maternal lines. Selection on uterine capacity has been used in animal breeding as a way to improve the litter size. A divergent selection experiment for uterine capacity was performed, obtaining large differences in the number of implanted embryos between rabbit lines after the first generation of selection. Segregation analysis suggests the presence of a major gene affecting the reproductive traits [1]. The major part of the differences between lines are due to a decrease in the number of descendents born in the low uterine capacity line, in which the embryo survival is lower [2]. The aim of this work was to test the *TIMP-1* gene as a candidate gene since it stands up as a target for the investigation of reproductive problems in humans. The rabbit *TIMP-1* gene structure and sequence were determined, including the proximal promoter region. We analyzed the parental generation of an F2 cross which consisted of 8 and 14 animals from the high and low uterine capacity lines, respectively. No polymorphism between lines was found in the screened regions (CDS, proximal promoter, exon 1, intron 1 and exon 2). However, real-time RT-PCR quantification of the *TIMP-1* mRNA showed significant differences between the two lines in the oviduct at 62 h of gestation, when rabbit embryos are located in this tissue.

Keywords: Gene structure, Uterine capacity, Embryo mortality, Expression, Oviduct

1 Argente et al. (2003) Genetics 163, 1061–1068.

2 Mocé et al. (2004) J. Anim. Sci. 82, 68–73.

### A1.22 A quantitative study on myogenic regulatory factors, Pax7 and Myostatin during postnatal porcine growth

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During embryonic development muscle progenitors cells migrate from the somite, originating primary and secondary myotubes due to the activation of a number of regulatory factors known as myogenic determination and differentiation factors (MRF). The presence of a third generation of myotubes has been described in the sheep, human and bovine during foetal life whereas in rats and pigs they appear shortly after birth and, only in porcine muscles, massively from the 3rd/4th weeks of postnatal life. To investigate the contribution of MRF, Myostatin and Pax7 genes to the establishment of postnatal hyperplastic mechanisms, we have used standard RT-PCR, quantitative real-time PCR, immunohistochemistry and cell culture experiments. Real-time PCR indicated a descending trend from 1 day towards 20 and 50 days after birth for MyoD, Myf5 and Pax7 while an increasing trend was detected for myostatin and myogenin. The biological significance of the decrease of Pax7 and the increase in myostatin mRNA at 20 and 50 days of age is probably due to the development of tertiary myotubes. Presumably, satellite cells are the cellular elements responsible for novel fibre development. A detailed assay on pig foetus is currently being performed in order to clarify if tertiary myotubes of the pig might be committed during the end of pregnancy or around birth.

Keywords: Growth factors, Satellite cells, Hyperplasia, Real-time PCR

### A1.23 Antiparasitic drug discovery: analysis of the brown dog tick salivary gland transcriptome

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Ticks and tick-borne diseases have a huge impact on animal health worldwide. Heavy tick burdens are associated with a reduction in weight gain and milk production in cattle and the wide variety of pathogens that ticks transmit also have debilitating effects. Control strategies rely heavily on the use of acaricides but excessive use has led to widespread resistance. Therefore new acaricides and/or novel drug targets are required to effectively control ticks. Data from a small sequencing project of 1500 expressed sequence tags (ESTs) from *Rhipicephalus sanguineus* salivary glands have been analysed. Using bioinformatics to convert the data into biologically relevant information, coupled with knowledge of tick physiology, several potential drug targets have been identified. We will present data from this project with particular emphasis on one of the targets, glutathione S-transferase.

Keywords: Drug discovery, Tick, EST analysis