

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****11.30 – 11.45am**

O2-1

MAMMALIAN CARBOXYLESTERASE GENES: SEQUENCES, STRUCTURES, PHYLOGENY AND EVOLUTIONRoger S Holmes^{1,2} and Laura A Cox¹

¹Dept of Genetics and Southwest National Primate Research Center, Southwest Foundation for Biomedical research, San Antonio, Tx 78227-5301 USA; ²School of Biomolecular and Physical Sciences, Griffith University, Nathan Qld 4111 Australia

Carboxylesterases (CES; E.C.3.1.1.1) exist as a family of enzymes which are capable of catalysing a broad range of hydrolytic and transesterification reactions. CES detoxifies organophosphate and carbamate compounds, catalyses several reactions of cholesterol and fatty acid acid metabolism and has been implicated in other metabolic processes in the body. The enzyme is predominantly localized in the endoplasmic reticulum, exhibits an N-terminal hydrophobic signal peptide consistent with a trafficking role through the ER and has a catalytic mechanism based on the activation of a serine residue at the active site by a histidine and aspartate (or glutamate) residue, as well an homologous α/β hydrolase fold tertiary structure.

We have studied the sequences, genetic variability, structures and phylogenetic relationships of baboon CES1 and CES2, which are highly homologous with the corresponding human enzymes, share key structural features reported for human CES1 and show family specific sequences consistent with the distinct subunit structures for these isozymes. We also used *in silico* studies to investigate human and mouse CES genes and obtained evidence for 5 families of CES genes. Several gene duplication events are proposed prior to mammalian evolution generating at least five of the CES genes on chromosome 16 of the human genome. Another gene duplication event is proposed during primate evolution, and several gene duplications are apparent during rodent evolution generating multiple mouse CES1-like and CES2-like genes arising separately within the rodent CES1 and CES2 gene lineages.

It is readily apparent that CES genes have undergone extensive gene duplication events over > 200 million years of evolution, for which the products have been retained within two linked clusters of CES genes. CES gene products show significant homologies in catalytic and regulatory domains, reflecting conservation of key residues for catalytic activity and the α/β hydrolase fold structures for these enzymes. Mammalian CES isozymes are however differentially expressed in tissues, exhibit different kinetic properties with biological substrates, have distinct tertiary structures as trimers-hexamers (CES1) and monomers (CES2), and may perform distinct roles in metabolism.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****11.45 – 12.00pm****O2-2****MODELING CARDIOVASCULAR DISEASE IN RAT BY ENU MUTAGENESIS**

Carol Moreno^{1,2}, Melinda R. Dwinell^{1,2}, Rebecca R. Schilling^{1,2}, Anne E. Kwitek^{1,2}, Andrew S. Greene^{1,3}, Richard J. Roman^{1,4}, Jozef Lazar⁵, David L. Mattson¹, Julian H. Lombard¹, Gregory D. McQuestion¹, Allen W. Cowley¹, Howard J. Jacob^{1,2/}

¹Department of Physiology, ²Human Molecular Genetics Center, ³Biomedical and Bioengineering Center, ⁴Kidney Disease Center, ⁵Department of Dermatology, Medical College of Wisconsin, Milwaukee, Wisconsin

The inability to generate knock-out (KO) rats has been a major limitation for studying gene function in rats. PhysGen, one of five Programs for Genomic Applications (PGA), uses a strategy that combines ENU mutagenesis to induce spermatid gene mutations with TILLING, a high-throughput assay which identifies mutations by enzyme cleavage of heteroduplex PCR product formation, followed by sequencing to confirm and identify KO rats at the genomic, rather than phenotypic, level. Using this method, we have identified point mutations in 112 selected genes in three different rat strains (Dahl S, BN, and FHH), which are then characterized physiologically for cardiovascular, pulmonary and behavioral traits. Many of these genes have been shown to have a cardiovascular effect. We have currently identified 65 mutations for 41 targeted gene, including one single codon deletion, seven nonsense, and 57 non-synonymous mutations of which 17 are predicted to impact protein function. Twenty-one mutations are predicted *not* to impact protein function and, therefore, are not physiologically screened. An ultrasound protocol assesses cardiac function; vascular function is studied in aortic rings; plasma levels 29 hormones, electrolytes and blood indicators are also measured, including a hemogram and lipid profiling. Blood pressure and protein excretion are also assessed. Some KO strains evidenced changes in the measured parameters, suggesting a functional role for these genes. For example, KO of the *Nr4a1* gene increased plasma white blood count cell, doubled the plasma lymphocyte counts, reduced blood monocytes, increased plasma cholesterol by 25% despite reducing body weight, and induced increased proteinuria.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****12.00 – 12.15pm****O2-3****DISEASE PORTALS AT THE RAT GENOME DATABASE**

Mary Shimoyama, George Kowalski, Stan Laulederkind, Rajni Nigam, Victoria Petri, Jennifer Smith, Jeff dePons, Dawei Li, [Simon Twigger](#), Anne Kwitek, Howard Jacob
Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, Wisconsin

The Disease Portals at the Rat Genome Database (RGD) provide a comprehensive platform for physiological genomics discovery through the integration of heterogeneous datasets into the context of the genome using multiple ontologies and sophisticated data mining and visualization tools. RGD serves a disparate community of users often defined by specific disease research areas. The Disease Portals provide both the novice and experienced user with easy access to a comprehensive, integrated knowledge base that can be tailored to the particular interests of the user. In addition, these initiatives define the focus and scope for data acquisition and curation projects. Current and planned components of the Disease Portals include: 1) comprehensive rat, human and mouse gene sets associated with disease, related phenotypes, disease pathways and related biological processes; 2) all rat QTLs related to disease, as well as associated mouse and human QTLs; 3) rat strains used as models in studying disease; 4) rat phenotype data including values and experimental conditions for model strains; 5) related references; 7) genome-wide view of disease genes and QTLs via Gviewer; 8) comparative maps of disease-related regions, 9) analysis and visualization of function and cellular localization of gene products. The current portals are designed to highlight genetic and genomic data generated from rat research in diseases related to the cardiovascular and nervous systems, with future portals covering other systems such as digestive, endocrine, immune, and musculoskeletal.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****12.15 – 12.30pm****O2-4****THE DEVELOPMENT OF CHROMATIN INTERACTION ANALYSIS USING PAIRED END DITAG SEQUENCING**

Yanquan Luo, Jun Liu, Hong Sain Ooi, Jianhua Liu, Chia-Lin Wei, Yijun Ruan
Genome Institute of Singapore, 60 Biopolis Street, Singapore 138672

Eukaryotic genomes are packed dynamically into higher order of chromatin structures, and the communication between distal chromosomal elements is known to be essential for control of many nuclear processes such as transcription regulation and DNA replication. Current methods for studying long-range interactions are limited in their scales and resolutions. To face this challenge, we have developed a new method for Chromatin Interaction Analysis using Paired-End diTag (ChIA-PET). This method enables unbiased and de novo mapping of 3-dimensional chromatin interactions at whole genome level. We used *Schizosaccharomyces pombe* as an experimental model to develop this approach and asked questions involved in long range interactions associated with transcription initiation complex. The current data suggests that the transcription of RNA polymerase III genes (tRNA genes and small rRNA genes) is highly coordinated and may play a role in organizing the 3-dimensional conformation of chromosomes in *S. pombe* nucleus.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****12.30 – 12.45pm****O2-5****THE EUROPEAN RAT TOOLS FOR FUNCTIONAL GENOMICS (EURATOOLS) CONSORTIUM**

Enrico Petretto¹, Stuart Cook¹, Jonathan Mangion¹, Herbert Schulz², Vladimir Kren³, Michal Prevenec³, Norbert Hubner², Timothy Aitman¹

¹MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London United Kingdom; ²Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany; ³Institute of Physiology, Czech Academy of Sciences and Centre for Applied Genomics, Prague

The European Rat Tools for Functional Genomics (EURATools) Consortium aims to determine the basis of genetic and phenotypic variation in mammalian biology by conducting functional genomics research in the rat as a model for human health and disease. Taking advantage of the availability of the rat genome sequence the EURATools Consortium will accelerate progress in mammalian genetics by means of five main research activities: (1) genome tools - annotation and functional analysis for genes and complex disorders, (2) nuclear transfer in the rat, (3) biological resources and toxicogenetics, (4) rat genome informatics and (5) gene expression analysis as a tool for gene identification. Through integrated and innovative genomics approaches we will facilitate the rapid identification of genes and pathways underlying complex rat disease phenotypes. For example, gene expression analysis with microarrays has proved remarkably successful when combined with linkage analysis to map the genetic determinants of gene expression (eQTL). Using this approach we generated genome-wide eQTL datasets in several tissues and employed Graphical Gaussian models on clusters of trans-eQTLs to identify gene regulatory networks associated with physiological phenotypes in the SHR rat. Analyses of these networks pointed to dysregulated regulatory pathways and allowed inferences to be made on the common underlying processes relevant to this model at the level of multiple tissues/organs. These data provide new insights into the control mechanisms for hypertension, insulin resistance and associated metabolic phenotypes that may be shared in common with similar disorders in humans.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****Monday October, 29****12.45 – 13.00pm****O2-6****GENETIC INVESTIGATION OF THE RAT APC PIRC MUTATION FOR LONGITUDINAL ANALYSIS OF COLON TUMORIGENESIS**JAMES AMOS-LANDGRAF¹, LAWRENCE KWONG², WILLIAM DOVE¹¹McArdle Cancer Research Laboratories, University of Wisconsin, Madison WI; ²Dana Farber Cancer Institute, Boston MA

We have established a model of human familial colon cancer that allows us to determine the fate of any colonic lesion with unknown neoplastic potential and investigate genetic effects of that fate. To monitor the neoplastic fate we have established in vivo endoscopic methods in the rat. The Storz Coloview system allows us to biopsy tumors at various times during tumor development and analyze both RNA and DNA. This longitudinal examination allows for a statistically robust platform to examine the genetic and genomic changes during tumorigenesis. We have extended our studies of the Pirc rat to investigate complex genetic background effects. Dominant resistance effects on tumor multiplicity have been discovered in crosses of the F-344-Pirc strain to inbred BDIX, Buf, and WF and to outbred SD. Recessive susceptibility effects have been observed in crosses to the inbred ACI strain. Coincident with the increase in colonic tumors was a decrease in the number of small intestinal tumors to 3-4 per animal, indicating a strong regional influence of a genetic modifier(s) in the rat genome. To map and manipulate dominant modifiers of the Pirc phenotype we will utilize the SNP genotyped F344xLE rat recombinant inbred lines maintained by Dr. Tadao Serikawa and the National Bio Resource Project for the rat in Japan. Thus, the biological and genetic resources of the laboratory rat enable longitudinal studies of colon cancer that complement those being pursued with the mouse and human.