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ABSTRACTS

Genomics of model organisms

082: Mining the human genome for components of cellular memory modules

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The epigenetic control of gene expression through global silencers like Polycomb (PcG) and activators like Trithorax (TrxG), group of proteins which form large multiprotein complexes is functional not only in Drosophila but also in the human genome. The interactions of these complexes are through fairly well defined *cis*-acting elements that are well characterized in the Drosophila system. Together the *cis*- and *trans*-acting factors form the components of the cellular memory modules. The sites of interaction of the classical PcG protein and its antagonist namely the TrxG protein are referred to as PcG/TrxG response elements, respectively (PRE/TRE) which mediates the inactivation or activation effects through chromatin remodelings complexes (PcG/TrxG) proteins, respectively. In humans, chromosome translocations involving global regulators result in pathogenesis leading to leukaemia of both B and T cell origin. There is a paucity of information on the *cis*-acting elements like PRE/TRE sequences in the human genome. We have taken a dual approach to mine the human genome for novel components of the global regulatory complexes that function through chromatin reorganisation. We have identified putative *cis*-acting elements through in silico analysis and these *cis* elements are validated by functional analysis in vitro and in vivo using transgenic Drosophila, as model system. We have demonstrated genetic interaction with some of the PcG and TrxG groups of proteins. We have detected interacting proteins in nuclear extracts from human cells in culture and modulation of reporter expression in transient expression assays in human cells in culture. We have identified putative PRE/TRE sequences in the human genome as the *cis* elements for chromatin remodelling complexes like PRC 1 and 2 and validated their function through in vivo analysis in transgenic models.

083: Investigating the influence of *cis*-regulatory elements in differential roles of the PAX258 gene family

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The paired box genes PAX2, PAX5 and PAX8, constitute a gene family that plays critical roles in interneuron specification, and in aspects of kidney, eye, ear and thyroid organogenesis. PAX2 is associated with renal coloboma syndrome and is involved in a spectrum of congenital abnormalities of the kidney and urinary tract. It has been associated with optic anomalies, is implicated in prostate cancer and plays a role in apoptosis resistance and angiogenesis. PAX8 is associated with congenital hypothyroidism and PAX5, which is inactivated in 32% of all B-progenitor acute lymphoblastic leukemias, is essential for B-cell differentiation. Although the PAX2/5/8 gene family has ancient origins, their functional coding domain is remarkably highly conserved. Functional redundancy, functional equivalency and interdependency have variously been implicated in several of the PAX258 expression domains, such that different members of the gene family can compensate for others. Therefore, rather than the coding sequence, *cis*-regulatory elements are strongly implicated in influencing differential and overlapping roles amongst the PAX258 gene family. Our genome wide comparative analysis has identified thousands of conserved non-coding elements (CNEs), with putative regulatory function. These cluster around developmental genes and occupy extensive upstream and downstream regions as well as intronic sequences. PAX2 alone is populated by almost 60 CNEs, covering a region of 363 kb. There are two copies of pax2 in teleosts, retaining a remarkable number of CNEs. Intriguingly despite a high percentage sequence identity, some duplicated CNEs show differences in their temporal and spatial up-regulation of a GFP reporter gene in zebrafish embryos. This yields a rich dataset for dissecting these elements experimentally and in silico in order to gain perspective on the language underlying tissue-specific expression patterns. In another approach we are using BAC recombineering in order to analyse entire regulatory loci in a GFP reporter system. PAX5 and PAX8 have comparatively simple regulatory landscapes, respectively, containing only 16 and 2 CNEs. We have generated recombineered BACs for both of these genes, with successful recapitulation of endogenous gene expression. Using a Cre-lox system to flip out selected CNEs we are now investigating the key role that these remarkable elements play as implicated by their conservation over 450 million years.

084: Genome-wide gene trapping identifies novel transcripts in zebrafish

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The unprecedented success in sequencing the whole genomes of organisms in recent times and the subsequent computational analysis of these genomes have yielded numerous candidate genes in any given biological process. While biological information for many of these genes has been documented, functional annotation of thousands of protein-encoding, non-coding RNA genes of unknown in vivo function still remains a major challenge to biology. Zebrafish (Danio rerio) provides a near ideal vertebrate genetic system to identify and annotate the biological role(s) of these novel genes because of their ex utero development, optical transparency of embryos, rapid embryonic development, short generation time and rapidly advancing genomic manipulation tools. This vertebrate model organism provides opportunities for investigating interesting questions in functional genomics. In order to begin understanding the complexity in the zebrafish transcriptome, we have conducted the genome wide Tol2 transposon based gene trapping studies. Tol2 transposon based gene trapping strategies have been developed in zebrafish for gene identification, gene discovery and insertion mutagenesis applications. We have developed a high-throughput gene-trapping assay in somatic tissues of zebrafish using the Tol2 transposon based gene traps. Using this technique we have characterized over 1,600 Tol2 transposase mediated de novo integration in the somatic tissues of zebrafish. Integrations of the gene traps were documented in well-annotated genes and transcripts of non-annotated genes in comparable proportions. However, approximately half of the total integrations mapped to un-annotated sequences in the zebrafish genome, suggesting that there are many more novel transcribed sequences (both coding and non-coding) yet to be discovered and annotated. Using a combination of molecular biology techniques we have demonstrated that over 400 such integrations in the un-annotated regions of the genome occurred in novel transcriptional units or hitherto undiscovered isoforms of known genes. Apart from that, though uniformly distributed in the zebrafish chromosomes, Tol2 based insertions show a bias towards integration in the first and last introns in the genes. Detail description of the global and local distribution of the Tol2 mediated integrations in the zebrafish genome will be presented.

085: Positional cloning identifies variants of A disintegrin-like metalloproteinase with thrombospondin motifs 16 linked to hypertension in a rat model and in humans

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Despite considerable research including recent genome wide association studies, identification of genetic elements that cause human essential hypertension has remained elusive. We investigated the genetic basis of hypertension in the Dahl Salt-sensitive (S) rat and identified a locus for blood pressure control on chromosome 1. Through sustained positional cloning efforts, this locus was mapped and isolated in a congenic segment less than 793 kb. The critical interval contains only two novel gene annotations. Complete sequencing of all predicted exons of both the novel rat genes indicated that there are two nonsynonymous variants associated with only one of these gene annotations, LOC306664. A full-length transcript consisting of 3666nt was expressed primarily in kidneys and in several other tissues. The expression of this transcript was not differential between that of the hypertensive parental and congenic strains. The predicted protein product of this locus is A disintegrin-like metalloproteinase with thrombospondin motifs 16 (Adamts16). One of the two nonsynonymous variants is within a furin-cleavage domain, while the other is within a thrombospondin domain. Results of the ongoing analysis of the functional consequences of these variants using both biochemical and integrated genomic approaches will be presented. Because Adamts16 is well conserved in rats and humans, we identified the homologous segment of our critical region in the Quebec Family Study wherein linkage evidence was additionally reported for systolic blood pressure. Interestingly, an Ala to Pro variant in codon 90 (rs2086310) of the human ADAMTS16 was significantly associated with human resting blood pressure. Overall, our report represents a high resolution positional cloning of a genetic element linked to hypertension in rats that is also associated with blood pressure in humans.

086: Cell-type specific transcriptional profiling in developing mouse embryos

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The analysis of gene expression in developing tissues and organs is a valuable tool for the assessment of genetic fingerprints during various stages of differentiation. By merging state-of-the-art technologies from the genomics field with classical molecular genetic approaches in mouse, we aimed to develop a robust strategy to isolate a cell population of interest from developing embryos within the context of time and space, for subsequent expression profiling studies. The initial study was carried out using a developmental control transcription factor that was tagged with the enhanced green fluorescent protein. Fluorescent activated cell sorting was employed to efficiently isolate the population of cells expressing the gene of interest from dissociated mouse embryos. These recovered cells were used for expression profiling with Illumina microarrays. By comparing the expression profiles of single and double knockouts, we identified 2,569 genes whose expression levels were changed at least 1.5-fold. In summary, we have developed a technology platform to isolate small population of cells from developing mouse embryos for expression profiling studies. This robust strategy should aid in the understanding of global regulatory network of developmental control genes and provide greater insight into the molecular basis of their role in development.

086: Genetic variation and population structuring of the domestic cat

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The domestic cat is one of the most popular companion animals. Large feral populations exist throughout the world, generally controlling vermin infestations, but sometimes wreaking havoc on indigenous ground mammal and bird populations. Approximately 50 breeds can be defined around the world, many of which share health problems found in humans. Random bred cats are even showing increases in incidence of complex diseases such as asthma, diabetes, and obesity. These breeds are having mainly developed in the past 150 years and little is known about the cat diaspora. Recent genetic studies have evaluated the domestication of the cat as well as the sub-structuring of cat breeds. Our own previous studies evaluated 39 microsatellite markers in over 1,000 cats, representing 19 cat breeds of the US and a variety of worldwide random bred populations. These early studies demonstrated that cat breeds are less sub-structured than dogs, but more so than humans. In addition, different regions of the world have distinct cat populations, and the breeds thought to be for those areas do in general show close relationships. This genetic research has now been extended to include several additional cat breeds as well as random bred cat populations from India, Iran, Jordan, Cyprus, increasing the study to over 2,000 cats. These populations focus on sites of early agricultural development, which are likely areas for early cat domestication. The same 39 microsatellite markers as the previous study have been evaluated in all additional individuals. To evaluate maternal contributions to population sub-structuring, approximately 400 bp of mtDNA control region has been sequenced in a subset of the 2,000 cat population, including nearly 1,000 cats. Also, 384 SNPs have been genotyped in a majority of the cats using Illumina GoldenGate assays and technologies. The SNPs were identified from the 2× sequence generated from an Abyssinian cat as part of the cat genome sequencing project. Bayesian clustering methods, network analyses, and several other phylogenetic inferences have been used to further define and demarcate the cat breeds and the random bred populations from around the world. These studies should help define the breeds and populations that should be considered for SNP validation for future genome-wide association studies in the cat.