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ABSTRACTS

Genomics of model organisms

550: Genomic dissection of the cellular exocytic machinery regulating airway mucus secretion

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Airway mucus is a component of the lung's innate immune defense that forms a protective barrier against inhaled particles and pathogens. Upon inflammatory stimulation, the airway epithelium undergoes a significant change in structure and function, due to increased mucin production by Clara cells. However, excessive mucin secretion or a failure of mucociliary clearance causes airflow obstruction and promotes airway infections. The precise mechanisms of mucin secretion and clearance in these protective and pathologic processes are not well understood at molecular, cellular, or tissue levels. To better understand these roles, we have engineered mouse hypomorph and conditional knockouts of key genes that regulate secretion, including Munc18b, a mammalian epithelial predominant isoform of the SM protein family, encoded by the Stxbp2 gene. In previous studies, absence of SM proteins has been shown to lead to a complete failure of secretion. We have found that Munc18b is localized at sites of secretion in the airway epithelium of mice. Furthermore, Munc18b is tightly associated with Syntaxin 2 and Syntaxin 3, but not Syntaxin 1 or 4. Syntaxins are key SNARE proteins facilitating secretion, and Syntaxin 3 regulates apical secretion in airway epithelium. Most importantly, we show that deficiency of Munc18b in Munc18b hypomorph mice, results in a decrease of induced mucin secretion. We find that embryonic stem cells express Munc18b and despite multiple crosses of Munc18b hypomorphs, a homozygous mutant has not been born. Together, these data indicate the critical role played by Munc18 proteins and suggest that their expression is tightly regulated. Epithelial isoforms of other exocytic proteins such as Munc13 and Synaptotagmin regulate specific aspects of mucin secretion, but are not as critically important as Munc18 proteins or Syntaxins. Our experiments with mutant mice lacking specific proteins allow us to analyze the molecular complexities of regulated airway epithelial cell exocytic secretion, and to test directly whether the presence of mucus on airway luminal surfaces is essential for lung clearance and host defense.

551: Gene expression profile in glucocorticoid-induced hypertrophied heart in rat

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Patients with various disorders including autoimmune diseases, allergic diseases and lymphoproliferaltive disorders are routinely treated with glucocorticoids. However, patients treated with excess of glucocorticoid exhibit a variety of symptoms including complications of cardiovascular system such as hypertension and arthrosclerosis. To understand the underlying mechanism, we performed microarray analysis of gene expression in rat heart after treatment with synthetic glucocorticoid, dexamethasone. Heart weight/body weight ratio was significantly increased with the duration of dexamethasone treatment up to 15 days compared to the vehicle treated control indicating the development of cardiac hypertrophy by glucocorticoid administration. Treatment of rat with dexamethasone significantly increased the expression of atrial natriuretic peptide, a molecular marker of cardiac hypertrophy which was unchanged when animals were co-administered with mifepristone, a glucocorticoid receptor antagonist. Microarray analysis revealed that the expression of 32 ion channel genes were altered in dexamethasone treated rat heart. Among these, L type calcium channels, sodium-calcium exchanger and calsequestrin were up regulated and phospholamban and nucleotide gated ion channels were reduced. Most of the genes those were altered in response to dexamethasone were restored to the level of control by mifepristone indicating the involvement of glucocorticoid receptor in mediating myocardial abnormality. Parameters such as ventricular remodelling, collagen deposition, myocardial contractile proteins and calcium channel proteins were examined. Dexamethasone also induced the collagen deposition and fibrosis in heart, which were correlated with the reduced expression and activity of matrix metalloproteinases I and 13. The results suggest that the increased collagen deposition, myocardial fibrosis and altered expression of the contractile proteins might be responsible for the malfunction of heart due to excess of glucocorticoid administration.

552: 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 Polycomb protein and its antagonist namely the Trithorax protein are referred to as Polycomb/Trithorax Response Elements respectively, (PRE/TRE) which mediates the inactivation or activation effects through chromatin remodeling 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 polycomb and trithorax 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.

553: A Drosophila systems model of locomotor plasticity relevant in epileptogenesis

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Kindling induced by pentylenetetrazole (PTZ) in rodents is the most popular chemical kindling model of epileptogenesis. In Drosophila adults, 7 days of PTZ treatment and 7 days of subsequent withdrawal respectively, caused a decrease and an increase in climbing speed. In this model, concomitant treatment with PTZ and antiepileptic drugs (AEDs) for 7 days showed sodium valproate (NaVP) and levetiracetam (LEV) as having an ameliorating effect on climbing speed decrease on 7th day and only LEV as having that effect on climbing speed increase on 14th day. When applied in the first four or last one day of chronic PTZ phase, LEV ameliorated climbing speed decrease on 7th day completely and partially, in that order, and NaVP the vice versa. NaVP thus showed a strong symptomatic and a weak prophylactic effect and LEV the reverse. Microarray expression profiling of fly heads after 12 h, 2 and 7 days of PTZ treatment showed downregulation of 23, 2439, and 265 genes, in that order. All three gene sets were enriched in synaptic remodelling, energy metabolism and transport related processes. NaVP and LEV caused upregulation of 170 and 12 genes which were enriched in energy metabolism and transport, and synaptic remodelling and energy metabolism, in that order. This suggested synaptic remodelling and transport as causal processes in development and in expression of locomotor alteration, respectively, and energy metabolism as having an intermediate role. Mining of available genetic, gene expression and proteomic data pertaining to mammalian models of epileptogenesis and epileptic patients supported the relevance of fly model. Locomotor effect of candidate gene silencing, small molecule treatment and dietary modifications demonstrated usefulness of the systems model in generating and testing hypotheses. Fly systems model offers a unique opportunity to identify disease, drug target, biomarker and pharmacogenomic candidates, and to screen potential therapeutic agents.

555: 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 (CAKUT). 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.

556: 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.

557: Lessons from the fruitfly: interaction between members of a conserved Wnt gene cluster

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Evolutionarily conserved gene clusters are important genomic assets for study of primal designs of genome evolution and functional significance of their persistent evolutionary selection. One such example is the members of Wnt gene family. The Human genome contains several Wnt clusters of which WNT6 and WNT10A genes on chromosome 2q35 are clustered 6.4 Kb apart and WNT10B and WNT1 are located 8.1 Kb apart on chromosome 12q13. These two Human Wnt clusters can be traced to an ancestral Wnt cluster which also gave rise to arthropod Wnt members. Drosophila has a 'Wnt cluster' comprising of wg-DWnt6-DWnt10 in section 27F on the left arm of the second chromosome. DWnt4, another member is located in close proximity to this cluster at 27E7. We have been particularly interested in investigating the functional relevance of these members as a cluster per se in the fruitfly. The wg is the paradigm of Wnts and DWnt4 is implicated in ovarian morphogenesis as well as in specifying dorso ventral specificity of retinal projections in the fly. The genes DWnt6 and DWnt10 have not been explored in detail as there are no classical mutant alleles and knowledge on their RNA interference and over expression phenotypes is lacking. Thus their exact role in the wild type is unclear. The conservation of DWnt6 and DWnt10 in the Drosophila genome contrasts with their apparent silence in mutagenesis studies raising the question of whether they can carry out redundant functions or whether each of them has a distinct role. We attempted to explore the biological potential of DWnt6 and DWnt10 by over expressing these genes in a DWnt4 lethal background using UAS-Gal4 system. We identified a new point mutant of DWnt4 that is an embryonic lethal which shows a phenotype affecting denticles in the ventral epidermis. Ectopic expression of DWnt6 and DWnt10 rescues DWnt4 phenotype in the denticles and also mimics the phenotype of DWnt4 ectopic expression in the wild type. The ability of DWnt6 and DWnt10 to substitute for DWnt4 activity in denticles suggests that the two genes are functionally analogous to DWnt4. Further, ectopic DWnt6 and DWnt10 in a DWnt4 lethal background produce extra denticles, a phenotype suggestive of an antagonistic function to that of wg. This is the first report that all members of this Wnt cluster have been shown to affect a common developing tissue, here the epidermis, however they do so differently.

558: Identification of potential drug targets in *Acinetobacter baylyi* using genomics approach

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Metabolic pathway analysis is increasingly becoming important for assessing inherent properties in biochemical reaction networks. The choke point strategy was implemented on the pathogen bacterial network of Acinetobacter baylyi. Potential drug targets are proposed based on the analysis of the top eight choke points in the bacterial network. A comparative study between the reported top 8 bacterial choke points and the human metabolic network was performed. Further biological inferences were made on results obtained by performing a homology search against the human genome. The study was successful in listing out the potential drug targets from these pathways which may be useful for the discovery of broad-spectrum drugs.

559: Differential gene expression in mice exposed to acute hypobaric hypoxia

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Atmospheric pressure and the partial pressure of oxygen decrease rapidly at increasing altitude (hypobaric hypoxia). Mammalian organisms have developed multiple protective mechanisms to adapt to hypoxia at the systemic, local and cellular levels. A broader understanding of hypoxia induced alterations could be achieved from the concerted application of functional genomics. Swiss albino mice were exposed to 0, 8, 10, and 12 h of hypobaric hypoxia (equivalent to 15,000 ft) in a decompression chamber and the temporal variation in gene expression in heart, kidney, and liver tissues were investigated by 15 k cDNA microarrays. Genes showing consistency between dye

swap experiments with up or downregulation more than twofold were selected and functionally classified according to the Gene–Ontology categories. Heart: The functional classification of the genes identified a number of pro-oxidants like Cyba, Xdh, Txnip, Ppp1r15b and antioxidants like Cat, Gpx1, Mt1, Mgst1 in the heart. Interestingly, the protein level of Cyba, a subunit of NADPH oxidase, which generates superoxide radical, was found to be markedly decreased in the acute hypobaric hypoxia exposed heart. Further, a significant reduction was observed in the level of reduced glutathione, indicating oxidative stress. Kidney: Hspa5, which plays a significant role in the ER stress response, along with other chaperones like Hsp90b1, Canx, Calr were transcriptionally downregulated in the kidney. Even the transcript levels of Ppp1r15a and Ddit3, which are important markers of unfolded protein response, were downregulated greater then twofold. Further, the splicing of Xbp1 mRNA decreased in response to acute hypobaric hypoxia, whereas transcription of the un-spliced mRNA increased. Hence, the ratio of spliced to un-spliced Xbp1 transcript which serves as a reporter on the level of ER stress along with decreased transcription of Hspa5, Ddit3 and Ppp1r15a indicated reduced ER stress in kidney. Liver: our study revealed $\sim$20 genes involved in cholesterol and phospholipid metabolism to be differentially regulated in the liver. Notably, by the 10th h several of the genes of sterol metabolism such as Srebf1, Insig1, Hmgcs1, Fdft1, Sqle and Hsd3b4 were downregulated greater then twofold suggesting that acute hypobaric hypoxia suppresses expression of genes involved in sterol biosynthesis in the liver. The present study thus provides a retrospective insight on the differential stress and metabolic responses in murine tissues in response to acute hypoxia.

560: Genetic analysis of γ-Glutamylcysteine synthetase enzyme involved in Glutathione biosynthesis in model organism *Saccharomyces cerevisiae*

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Glutathione(GSH) biosynthesis is a two-step ATP dependent process. The first step is the rate limiting step and is catalyzed by γ-glutamylcysteine synthetase (γ-GCS) ligating glutamic acid and cysteine to synthesize γ-glutamyl-cysteine. The enzyme is under strict transcriptional and post-translational regulation. The importance of GSH homeostasis has been implicated in various diseases conditions. Thus, understanding the rate-limiting step of GSH biosynthesis catalyzed by γ-GCS is important and has prompted studies on the structural/functional aspects of γ-GCS. In the absence of a crystal structure of the γ-GCS enzyme present in yeasts, mammals, we have initiated a mutational analysis to understand the role of different amino acid residues in the functioning of yeast γ-GCS enzyme. Our focus has been on the role of cysteines in regulating enzyme activity in response to changing redox conditions of the cell and also amino acids residues present in the putative cysteine binding pocket of γ-GCS enzyme. Our studies, using genetic suppressor analysis of some of the non-functional γ-GCS mutants has revealed some interesting new insights on the role of cysteines in the functioning of the enzyme.

561: Study of the gamma glutamyl cycle in yeast

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Glutathione is the major low-molecular weight thiol compound present in almost all eukaryotic cells at intracellular concentrations ranging from 0.1 to 10 mM. The gamma-glutamyl cycle carries out the synthesis and degradation of glutathione through six enzymatic steps. This cycle is also involved in transport of amino acids in mammalian cells. In Saccharomyces cerevisiae, two enzymes of the gamma-glutamyl cycle namely gamma-glutamyl cyclotransferase and 5-oxoprolinase, the last two enzymatic steps in the cycle could not be detected, and thus this cycle has been thought to function as a truncated cycle in yeasts. We are investigating the gamma-glutamyl cycle in yeast, and to understand why yeasts might have evolved a truncated cycle. The possibility that the alternative pathway of glutathione degradation in *S. cerevisiae* that has recently been discovered in our lab might be playing a compensatory role leading to a truncated pathway is being investigated. These results and hypothesis on the functioning of the gamma-glutamyl cycle in yeast will be presented.

562: Homocysteine induces mitochondrial stress mediated cell death in *Saccharomyces cerevisiae*

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An elevated level of the thiol amino acid homocysteine has been implicated as an independent risk factor for cardiovascular diseases and has also been associated with various other complex disorders like schizophrenia, Alzheimer's disease, neural tube defect, diabetes, etc. We believe that a single amino acid's association with so many diseases warrants its capacity to alter basic cellular processes/pathways. We used the budding yeast *Saccharomyces cerevisiae* as a model system to mechanistically understand the basis of homocysteine induced pathogenecity. We had earlier shown that homocysteine induced growth defect in wild type yeast strain is due to endoplasmic reticulum stress (Kumar et al. 2006 Biochem J). In this study we used str4Δ strain (where conversion of homocysteine to cystathionine and cysteine is blocked) to evaluate the effect of homocysteine and its precursors S-adenosyl homocysteine (SAH) and S-adenosyl methionine (SAM) on yeast growth and global gene expression with a view to mechanistically understand their role in vascular disease process. Vascular cells lack cystathionnne β-synthase (str4Δ in yeast), an enzyme that converts homocysteine to cystathionine. We found that both homocysteine and SAH, but not SAM, inhibits the growth of str4Δ strain in a dose dependent manner. Although SAM abrogated the inhibitory effect of SAH as has been previously reported, it failed to rescue the inhibitory effect of homocysteine indicating that increase in the SAM/SAH ratio is sufficient to overcome SAH mediated growth defect but not homocysteine induced growth inhibition. Transcriptional profiling of the str4Δ strain exposed to SAM, SAH and homocysteine individually or in combination revealed that the profiles of SAH and homocysteine were grossly dissimilar indicating that the toxicity induced by homocysteine and SAH may be via distinct mechanisms. We also found that SAH but not homocysteine induced G1/S cell cycle arrest and this was overcome with the addition of SAM. Unlike the wild type yeast strain, addition of homocysteine to str4Δ did not lead to endoplasmic reticulum stress. Rather, homocysteine was found to impair the mitochondrial membrane potential. It also led to mitochondrial fragmentation and apoptosis as revealed by annexin V staining and TUNEL assay. Thus, in str4Δ strain where the transsulfuration pathway is absent, the mechanism of homocysteine induced growth inhibition was different from that in wild type strains.

563: Role of intracellular calcium in *Drosophila* larval growth and viability: a microarray analysis

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Intracellular Ca^{2+} release through the Inositol 1,4,5-triphosphate receptor (InsP3R) is a feature of all multicellular organisms in which it shapes the temporal and spatial aspects of calcium signalling in cells, affecting several developmental and physiological processes. Mutants in the InsP3R gene of *Drosophila* (itpr) exhibit a range of defects including altered wing posture, increased spontaneous firing and loss of the rhythmic flight patterns in response to an air puff stimulus, grossly leading to loss of flight behavior. Some heteroallelic combinations of itpr mutants are lethal and exhibit growth defects. One such combination is itpr sv35/ug3 (Joshi et al. 2004). Previous work from our group has shown that InsP3R function can be compensated by altering Sarco-Endoplasmic Reticulum Calcium-ATPase (SERCA) activity (Banerjee et al. 2006). Our aim is to understand the role of itpr gene in larval growth and viability and to decipher its cross talk with other signalling pathways. To address this question (a) an UAS-itpr + transgene was over expressed in the *Drosophila* insulin like peptide producing cells of the larval lethal itpr mutant (itpr sv35/ug3), and (b) a dominant mutant for the Sarco-Endoplasmic Reticulum Calcium ATPase gene (Kum170) was introduced into the larval lethal itpr mutant (itpr sv35/ug3) background. We observed partial rescue of itpr mutant phenotypes in Dilp: itpr + and Kum170 rescue conditions. In order to understand the molecular basis of these rescues, we have carried out microarray screens. Of the total 20056 genes present on the microarray slide 1,212 genes were down regulated and 1,346 genes were up regulated in itpr sv35/ug3 animals. Among these we have identified a number of genes whose expression levels are rescued in both rescue conditions. These genes are categorized on the basis of their function and their involvement in different signal transduction pathways. We find several genes that fall in the immune response category. Validation of these significantly regulated genes by RT-PCR and Quantitative RT-PCR is in progress along with the functional validation by genetic tests. We are also studying the expression pattern of selected genes at different developmental stages. Results from these experiments will be presented.

References

1. Joshi R et al. (2004) Genetics 166:225–236
2. Banerjee S et al. (2006) J Neurosci 26:8278–8288

564: In silico identification of novel DOF (DNA binding with one finger) genes from Sorghum bicolor using comparative genomics approach

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The dof (DNA binding with one finger) is a plant specific transcription factor extensively studied in diverse plant species and involved in gene regulation of processes restricted to the plants. The dof domain contains highly conserved amino acid sequence involved in DNA binding at *N*-terminal and diverse amino acid sequences outside the dof domain. Based on in silico studies numbers of putative dof genes have been identified in crops. The availability of complete genome sequences of rice provide an opportunity to fish out novel dof genes from other crops based on comparative genomic approach. In the present investigation an attempt has been made to fish out the putative full-length dof genes in Sorghum bicolor based on the information of dof genes in rice. Two full length dof genes (Sbdof1 and Sbdof2) were characterized from the available BAC clones (AY661659, AC169375) of Sorghum bicolor. The sequence of putative dof genes were subjected to homology search with NCBI using BLAST. These were further analyzed by bioinformatics tools namely GENESCAN and FGENESH for fishing out the probable gene and the putative CDS and protein sequence. Protein domain classification of the putative genes has been characterized by INTERPROSCAN and CDD which shows identity with Zinc finger family of proteins. The sequences showing maximum homology with SbDof1 and Sbdof2 were subjected to multiple sequence alignment using ClustalW and a phylogenetic tree was constructed by MEGA3.1 tool. The predicated *cis*-regulatory region (−1,000 bp region) of both the genes were analyzed for conserved *cis*-regulatory elements based on PLACE and PLANTCARE databases.The diverse functions attributed to dof genes are due to interaction of transcription factors with different conserved sequences in the promoter regions of the respective genes. The promoter analysis of putative Sbdof1 and Sbdof2 genes reveals its function in seed development and defense response, respectively. Some of the important *cis*-regulatory elements common to both genes were RY-element, Skn-1_motif, Sp1, TATA-box, CAAT box and G-box while 5UTR Py-rich stretch, ATC-motif, CCAAT-box, GCN4_motif, MNF1, TGA element, TA rich and Circadian is present exclusively in Sb Dof1. In case of SbDof2 *cis*-element namely A-box, ABRE, TGACG motif, GC motif and CAT box were observed. The protein sequences subjected to motif scan revealed the presence of glycine rich region profile in SbDof1 while proline rich region profile was observed in SbDof2.

565: 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 (EGFP). Fluorescent Activated Cell Sorting (FACS) 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.

566: 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 substructuring of cat breeds. Our own previous studies evaluated 39 microsatellite markers in over 1,000 cats, representing 19 cat breeds of the United States and a variety of worldwide random bred populations. These early studies demonstrated that cat breeds are less substructured 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.

567: Gene expression profile in hyperthyroid induced hypertrophied heart in rat

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Hyperthyroidism is known to cause abnormal heart functions, cardiac hypertrophy and heart failure. To understand the molecular mechanism of cardiac malfunctions in hyperthyroid condition, gene expression profile in thyroid hormone treated rat heart was identified by cDNA microarray analysis. About 40 genes were found to be differentially regulated by thyroid hormone. Further analyses by Western blotting, Northern blotting and real-time quantitative RT-PCR of some of the genes confirmed the microarray results. The thyroid hormone-altered genes encode various types of proteins related to metabolism, matrix and cytoskeletal structures, growth factors, transcription receptors, Ca^{2+}-channels etc. Among the altered genes, about 80% were negatively regulated by thyroid hormone. Interestingly, co-treatment with a potent antioxidant, *N*-acetyl cystine caused restoration of some of the down regulated genes in hypertrophied heart. These include insulin responsive glucose transporter type 4 (Slc2a4), Cu–Zn-SOD (Sod1) and the MEF2 transcription factor family. The results show that oxidative stress plays a major role in inhibiting gene expression in hypertrophied heart in rat. The present finding will be important for the development of therapeutic approaches to cardiovascular disease.

568: G-protein coupled signaling in *Ciona intestinalis* : a comparative genomics perspective

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Ciona intestinalis, a protochordate with a compact genome has an ancestral complement of many diversified gene families of vertebrates and is a good model system for studying chordate to vertebrate diversification. G-protein coupled receptors (GPCRs) are a large family of integral transmembrane receptor proteins devoted to signal transduction. An *in silico* comparative genomics analysis of the *Ciona* repertoire of GPCRs and G-proteins with those of human and other vertebrates provides insight into the evolutionary origins of the GPCR signaling system in vertebrates.

We have identified 169 gene products in the *Ciona* genome that code for putative GPCRs. Phylogenetic analysis reveal that *Ciona* has representatives belonging to the five major GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled, Secretin) families of GPCRs apart from possessing remote non-chordate homologs. Nearly 39% of *Ciona* GPCRs had unambiguous orthologs of the human GPCRs. A significant number of non-orthologous genes include a lineage specific gene expansion of one group of Rhodopsin receptors that possess a novel domain organization hitherto undetected in the metazoa. Our analysis also identified unambiguous *Ciona* orthologs of vertebrate G-proteins and G-protein associated effector proteins. Our studies thus suggest that the tunicates possess the basic ancestral complement of the GPCR signalling pathway and that this evolutionarily conserved pathway predates the split of the chordates from the vertebrates in evolution. The observation of homologs to many GPCRs related to vertebrate heart and neural tissue physiology raises an interesting possibility where studies addressing *Ciona* GPCR biology could be meaningfully extracted to assess the functions of complementary vertebrate genes. A comparative analysis of the repertoire of GPCRs and G-proteins in *Ciona* with that of human will be presented.

569: Comparative transcriptomic analysis identifies functional conservation of estrogen responsiveness between zebrafish and human cancer cell lines

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Estrogen plays a key role in vertebrate development and reproduction. Long-term exposure of male fishes to estrogen induces feminization and short term exposure results in genome-wide changes in the expression of estrogen responsive genes. These global effects are reminiscent of those observed in hormone-dependent estrogen receptor (ER)-positive human cancer cells. In this study, our aim was (a) to identify estrogen responsive genes in zebrafish and (b) to determine the extent of conservation of estrogen responsiveness between zebrafish and human cancer cell lines to discern the core mechanisms of estrogen response in developmental and disease processes. Using a high density zebrafish oligonucleotide microarray, we have identified estrogen responsive ER-regulated genes in whole fishes, by treating male zebrafish with estrogen and anti-estrogen, ICI 182,780. We have discovered a cohort of estrogen responsive genes in zebrafish and validated a subset of these genes by quantitative PCR. Ingenuity Pathway Analysis (IPA) of zebrafish estrogen responsive genes identified significant enrichment of genes involved in specific biological processes such as cell cycle, cell proliferation, cancer, and DNA repair. Comparative analysis of estrogen responsive genes identified in human cancer cells (MCF7, T47D and Ishikawa) and those identified in zebrafish were carried out to map the gene to gene conservation and pathway/gene ontology based conservations among them. Gene to gene conservation analysis showed low percentage of conservation between zebrafish and human cell lines and also among the human cell lines. Pathway and gene ontology based analysis has revealed a significant conservation in estrogen responsiveness between fish and human cell lines as well as among the cell lines in a number of biological functions and pathways such as cell cycle, cancer, and cell proliferation. Furthermore, ER binding site analysis showed an enrichment of binding sites in the human homolog of zebrafish estrogen responsive genes, suggesting the conservation of regulatory mechanisms and potential responses across species. Comparative analysis of estrogen responsiveness between zebrafish and human cancer cell lines has identified the conservation of core target genes and a number of biological functions/pathways. Such conservation indicates that zebrafish is an ideal vertebrate model for estrogen related research, including functional studies and screens for small molecules with agonistic and antagonistic effects on ER.

570: Analysis of the mechanisms of differential facultative chromatin organization in a model system

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The role of chromatin in epigenetic regulation is increasingly being appreciated since it is important in determining the accessibility of DNA for vital cellular processes. The investigation of the mechanisms of non-Mendelian inheritance of genetic disorders so far, indicates the lack of a universal mechanism. Therefore the analysis of systems that resort to epigenetic regulation for developmental decision provides the insight into possible mechanisms that can bring about differential regulation of homologous chromosomes for divergent developmental pathways. The mealybug, a coccid insect system offers such a system replicating the sex-specific facultative inactivation of 50% of its genome, similar to X-chromosome inactivation in female mammals. Our studies so far, strongly suggest differential chromatin organization as a possible mechanism of genomic imprinting in mealybugs. We have demonstrated an unusually compacted nuclease resistant chromatin (NRC), specifically in male mealybugs of two species, *Planococcus lilacinus* and *Maconellicoccus hirsutus*. The association of NRC with the nuclear matrix renders it nuclease resistant while its dissociation leads to nuclease susceptibility. We have analysed the nuclear proteome of the male and female mealybugs to identify differentially abundant nuclear proteins by DIGE (Difference in Gel Electrophoresis). In addition, we have carried out a comparative analysis of histone modifications in the two sexes and find that the occurrence of H3K27 trimethylation is significantly higher in male nuclear matrix as compared to that in female mealybugs. In summary, there is a strong evidence for differential matrix association leading to sex specific unusual chromatin organization to be a correlate of genomic imprinting in mealybugs with a strong possibility of having a causative role as well.

571: Community annotation of the zebrafish genome: a wiki solution

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An enormous amount of information on a genomics scale is available for Zebrafish (Danio rerio), which is a well-studied model organism for Human diseases. However, a majority of this annotation is scattered in obscure data sources, which are not easily amenable for large-scale and integrative analysis. To integrate the zebrafish centric genomics information into a unified resource we have developed 'FishMap'—A platform for storage, display and retrieval of genomic information of zebrafish. This database provides a centralized, web accessible, integrated resource for common access to this data while allowing sophisticated data mining queries. The datasets in FishMap have been methodically collected from various resources and supplementary information of publications and mapped to the zebrafish genome. The data is organized into nine major sections, which include Comparative Genomics, Mapping and Sequencing, Gene and Gene Predictions, Expression and Regulation, and Variation and Repeats. In addition to an integrated genomics database, FishMap aims to provide shared genomics resources for community annotation of the zebrafish genome on the lines of the 'wiki' called the 'Zebrafish GenomeWiki'. Members of the zebrafish community can annotate, comment and edit existing data sets using the Zebrafish GenomeWiki portal. The Zebrafish GenomeWiki will permit community experts and individual investigators to add the latest genomics information and discuss alternative annotations there by speeding up the fine annotation the zebrafish genome. FishMap is built on the Gbrowse, which is a part of the Generic Model Organism Database Consortium Project. The database is amenable to programmatic access through the Distributed Annotation System (DAS) as well as BioMoby protocols, thus making it a central community resource that can be integrated with existing data mining and analysis workflows. We hope FishMap together with the Zebrafish GenomeWiki would be an integral resource for community participation in zebrafish genomics. The resource is freely available at URL: http://miracle.igib.res.in/fishmap or http://fishmap.igib.res.in/.

572: Transcriptional profile of immune response genes in mammals at high altitude

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High-Altitude (HA) environment is characterized by hypobaric hypoxia. Ascent to HA (>2,500 m) or exposure to hypoxia, among various biological systems also affects specific components of the immune system. We used Affymetrix bovine oligoarrays to compare the transcriptional profile of *Bos taurus* at HA and sea level. It was also investigated whether the observed changes were exclusive to *B. taurus*. Since, the MHC region has an important role in immune responses, we explored the changes in the transcript levels of class-II MHC genes in healthy male human volunteers ($n = 5$) exposed to hypobaric hypoxia for 24–72 h at 11,000 ft. Semi quantitative RT-PCR was used to explore the mRNA levels in human subjects. In the *B. taurus*, more than twofold downregulation of genes involved in immune response and inflammation was observed. The genes which were downregulated are: IFI30, CD164, CTSC, IL18, IL15, ANX A11, SDF1, CXCR4, TLR6, TNFRSF1A, PLA2G7 and BLA-DQB. Quantitative PCR confirmed the downregulation, indicating that hypoxia suppresses immune response. RT-PCR showed complete downregulation of transcript level of the class-II MHC genes in the healthy human volunteers.

The results are thus suggestive of transcriptional downregulation of immune response genes at HA as an acclimatory response in bovine and human subjects.

573: Study of the role of genetic heterogeneity in response to low dose ionizing radiation using mice as model system

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In the recent years, studies on the effect of low dose ionizing radiation (LDR) have become a matter of intense research because of constant exposure from natural sources and increased therapeutic and diagnostic usage of LDR. It has been shown that transcriptional responses at low doses are quantitatively and qualitatively different from that of higher doses of radiation and differ in tissue as well as sex specific manner. DNA microarray technology has provided a means to study global gene expression changes in response to LDR. Genetic susceptibility of an organism appears to plays a significant role in overall radiation effect. Identification of patterns of genetic and epigenetic factors may not only lead to identification of markers for exposure, but may also provide insight into responses of organisms to radiation exposure. Radiation sensitivity studies in various mouse strains like BALB/c and C57BL/6 have shown BALB/c to be sensitive to the lethal effects of radiation and to the development of various types of radiation induced solid tumors. In the present study we have used fractionated whole-body low dose gamma-irradiation of 20 cGy to study the effect of LDR on the gene expression profile of BALB/c and C57BL/6 liver tissue using microarray. Significant differences in the expression of the genes involved in signal transduction pathways like insulin receptor mediated signalling, TGF-β (Transforming growth factor β) receptor mediator signalling and G-protein coupled receptor mediated signalling have been observed in these two strains in response of radiation. Differential expression of the genes involved in inflammation and other immune responses were also observed in response to radiation. A large number of genes involved in focal adhesion and cytoskeleton development also showed significant differences in the gene expression pattern. We have also studied the effect of low dose exposure on the gene expression profile of F1 progeny of these two strains of mice. Sex-specific reciprocal crosses were set between the two strains to study parent of origin specific responses. Analysis of gene expression pattern showed that F1 progeny shows parent of origin specific effects, clearly indicating the involvement of genetic and epigenetic factors. Our data suggests possible involvement of individual genetic background and immunological radio sensitivity in the two mice strains and parent of origin specific effects in F1 progeny in response to LDR.

574: Evaluation of potential risk on mammalian embryos of nanoparticles introduced systemically on the mother during pregnancy

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The increasing use of nano-materials in industry and in everyday life has raised concerns as to their potential risks when inhaled or upon systemic introduction into the body. Their adverse pulmonary effects have been documented while information is lacking as to their short-term or long term effects if any, on embryos and foetuses upon systemic exposure of the mother. To evaluate their potential embryo toxic effects in a model organism, we utilized two types of nanoparticles, single-walled nanocrystals (SWCNTS) and Quantum Dots (QDs), and introduced them systemically into pregnant mice at various gestational stages. Our results reveal that midgestation embryos of dams injected systemically with QDs either at early or late embryonic stages developed well into more advanced stages without exhibiting any gross developmental abnormalities, or reduction in average litter size when compared to controls. We found that QDs were lodged in the placenta but we failed to detect any in the embryo proper. While in vitro, SWCNTs but not QDs were cytotoxic to cells upon prolonged exposure our in vivo results so far suggest that at least for the time-points studied, the mammalian placenta served as a fairly robust barrier that was able to act as sieve to prevent the introduction of nanoparticles systemically introduced into the mother from entering the embryos.

575: Genomic mechanism of levetiracetam action in a *Drosophila* systems model

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Despite its increasingly widespread clinical use in treating different epilepsies and therapeutic potential in various other neurological and neuropsychiatric conditions, levetiracetam (LEV) remains an enigma. Although it is known to specifically bind to the synaptic vesicle protein, SV2A, the mechanism of action of LEV is not yet clear. Here, we used a newly developed Drosophila systems model of brain plasticity relevant in epileptogenesis and antiepileptic drug (AED)

screening to identify genes, biological processes and biochemical pathways underlying LEV's mode of action. In the newly described Drosophila model, chronic treatment with pentylenetetrazole (PTZ) cause climbing speed deficit. Development of this deficit is blocked by LEV. Genome level expression profiling of fly heads associated with time series of PTZ and LEV treatments separately has suggested that LEV acts through upregulating synaptic remodelling and energy metabolism. We present here time series of microarray expression profiles associated with a combined PTZ and LEV regimen that was shown not to result in climbing speed deficit. Comparison of these expression profiles with PTZ alone and LEV alone profiles described earlier suggested that synaptic remodelling and energy metabolism are indeed primarily and secondarily associated with prophylactic action of LEV, in that order. Further, transcriptomic analysis provided evidence that glutamate metabolism is the initial pathway involved in LEV's action. Cell non-autonomous RNAi against Got2 caused climbing speed deficit that was ameliorated by LEV. This supports the candidacy of Got2 as the crucial target in the mode of action of LEV. Overall, our findings are consistent with LEV's known binding to a synaptic vesicle protein in rat and human brain and its association with, in a rat model of epileptogenesis, changes in levels of proteins regulating glutamatergic excitatory synaptic transmission efficacy.

576: Insertional mutagenesis screen in Zebrafish using gene breaking trap approach

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Zebrafish is a popular model organism offering a powerful combination of low cost, rapid in vivo analysis and complex vertebrate biology. In spite of the advances in zebrafish genomics, majority of zebrafish genome remains unannotated and uncharacterized thus leaving their functions unknown. To fill this gap we would like to carry out a large-scale insertional mutagenesis screen in zebrafish employing the transposon based gene breaking trap (GBT) approach.

The GBT offer several advantages in screening and molecular characterization of the tagged loci over standard insertional mutagenesis approaches. Since the GBT is not expression dependent, it can trap and mutate genes that would not be isolated in classical phenotypic-driven insertional mutagenesis screens. In addition the GBT also offers the possibility of establishing a sequence-based database of insertional alleles in genes of high biological interest and in parallel, create a resource of mutants in genes that might have adult phenotype as well as isolate mutation that result in the more traditional embryonic and larval phenotypes. We have initiated a large-scale insertional mutagenesis screen in zebrafish using the Tol2 transposase mediated GBT vector. To date we have co-injected Tol2 transposase and GBT in approximately 2,000 embryos and are growing them to adulthood for mutation screening. The results and updates from this study will be presented at the meeting.

577: Paradoxical signature of antiepileptic drugs in a *Drosophila* genomic model

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A *Drosophila* systems model of chronic pentylenetetrazole (PTZ) induced brain plasticity relevant in epileptogenesis and antiepileptic drug (AED) screening has recently been developed. Whereas the AEDs sodium valproate (NaVP) and levetiracetam (LEV) show effectiveness, other AEDs, namely, ethosuximide (ETH), gabapentin (GBP) and vigabatrin (VGB), have not been found effective in this model. Genome level expression profiling of fly heads has suggested that downregulation of biological processes related to transport/localization and energy metabolism, and synaptic remodelling and energy metabolism are central in the fly PTZ model. It has also suggested that upregulation of the above processes underlie mode of action of NaVP and LEV. Here, we present transcriptomal mode of action of ETH, GBP and VGB in the fly model. These drugs were mainly found to downregulate genes. Significant overlap was observed among genes downregulated by ETH, GBP and VGB. Importantly, significant overlap was also observed between each of these gene sets and genes downregulated by PTZ and genes upregulated by NaVP and LEV. Further, ETH and GBP genes overrepresented processes related to synaptic remodelling, energy metabolism and transport/localization. Furthermore, ETH, GBP and VGB expression profiles showed similarity with an available proteomic profile of human temporal lobe epilepsy. Our results therefore suggest that ETH, GBP and VGB might be epileptogenic. Seizures are known to be vulnerable to aggravation by AEDs. Broad-spectrum AEDs such as NaVP and LEV are however, less likely to exacerbate seizures. Our results are thus consistent with clinical evidence. Also consistent is our observation that ETH, GBP and VGB affect structure and function related to vision. The new Drosophila systems model offers a unique opportunity to dissect mechanisms of action of drugs used in treating neurological and neuropsychiatric conditions in general and epilepsy in particular.

578: Application of comparative genomics for identification of genes involved in plant stress from Octodecanoid and Jasmonic acid pathway of Rice and *Arabidopsis thaliana*

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Many plant genes that respond to environmental and developmental changes are regulated by jasmonic acid, which is derived from linolenic acid via the octadecanoid pathway. Linolenic acid is an important fatty-acid constituent of membranes in most plant species and its intracellular levels increase in response to certain signals. The octadecanoid pathway is a reasonably well-characterized biosynthetic pathway for the production of the phytohormone jasmonic acid (JA), an important hormone for induction of defense genes. JA is synthesized from alpha-linolenic acid, which can be released from the plasma membrane by certain lipase enzymes. For example, in the wound defense response, phospholipase C will cause the release of alpha-linolenic acid for JA synthesis. In the first step, alpha-linolenic acid is oxidized by the enzyme lipoxygenase. This forms 13-hydroperoxylinolenic acid, which is then modified by a dehydrase and undergoes cyclization by allene oxide cyclase to form 12-oxophytodienoic acid. This undergoes reduction and three rounds of beta oxidation to form jasmonic acid. Here in this research work we report we identified important genes i.e., Lipoxygenase genes (lox1-EU725461, lox2-EU725462, lox3-EU700314) in Oryza sativa Nipponbare japonica cultivar using Arabidopsis thaliana Columbia as model plant and we design primers for full length gene for verification in Pusa basmati-1 Indica cultivar. We have successfully identified these three genes and validate using bioinformatics tools and databases. We also check their expression during developmental changes in Pusa basmati-1.

579: Creation and characterization of Wdr13 gene deficient mouse

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The WD-repeat proteins are found in eukaryotes and play an important role in the regulation of a wide variety of cellular functions such as signal transduction, transcription, and proliferation. These proteins provide platform for protein-protein interaction. The WD repeat, to which the family owes its name, is a conserved motif of nearly 40 amino acids that often ends with the di peptide Trp-Asp (WD). To determine the functional significance of this gene in mammals, this gene was cloned and characterized in mouse. Like its human counterpart, mouse Wdr13 gene is localized on the X chromosome and encodes a WD-repeat protein of 485 amino acids. We have deleted this gene in mouse embryonic stem cells through homologous recombination. Knockout mice were generated after injection of targeted ES cells into blastocyst. Knockout mice were viable and fertile. Since Wdr13 gene expresses predominantly in testis, we analysed various sperm motility and fertility parameters. We did not find any significance difference in various sperm parameters of knockout mice in comparison to wild-type animals. Currently we are doing microarray analysis from testis of knockout mice and its wild type littermates.

580: 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 1600 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 (ESTs) 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.

581: Boundary element separates the differentially expressing genes myoglianin and eyeless on the fourth chromosome of *Drosophila melanogaster*

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Regulation of genes in eukaryotes is dependent on large number of regulatory elements. Chromatin domain boundaries are a class of elements that do not allow cross talk between regulatory elements of neighboring genes and are important for proper expression of genes. Using simple bioinformatic approach, we have identified a new boundary element, ME boundary, on the fourth chromosome of Drosophila melanogaster which separates two differentially expressing genes, namely, myoglianin and eyeless. Myoglianin is predominantly expressed in the muscle tissue whereas eyeless is predominantly expressed in the central nervous system and the eye. Taking transgenic approach we show that ME intergenic region acts as a boundary both in the embryonic and adult stages. Using both biochemical and genetic approaches we identified BEAF and TRL as the major *trans* acting factors responsible for the boundary activity of this element. Some of the proteins that had marginal effect on the boundary function are ASH1, BRM, and PC. Our results demonstrate that a rational approach can be applied to identify functional boundaries that play important role in differential expression of closely spaced genes.

582: Pgt1, a glutathione transporter from the fission yeast *Schizosaccharomyces pombe*

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The *Schizosaccharomyces pombe* ORF, SPAC29B12.10c, a predicted member of the Oligopeptide transporter family (OPT), was identified as a gene encoding the *S. pombe* glutathione transporter (Pgt1) by a genetic strategy that exploited the requirement of the cys1aΔ strain of *S. pombe* (that is defective in cysteine biosynthesis) for either cysteine, or glutathione, for growth. Disruption of the ORF in the cys1aΔ strain led to an inability to grow on glutathione as a source of cysteine. Cloning, and subsequent biochemical characterization of the ORF revealed that it is a high affinity transporter for glutathione ($K_m = 63$ μM) that was found to be localized to the plasma membrane. The transporter was specific for glutathione, as significant inhibition in glutathione uptake could be observed only by either reduced or oxidized glutathione, or glutathione conjugates, but not by dipeptides or tripeptides. Furthermore, although glu-cys-gly, an analogue of glutathione (γ-glu-cys-gly), could be utilized as a sulphur source, the growth was not Pgt1-dependant. This further underlined the specificity of this transporter for glutathione. The strong repression of $pgt1^+$ expression by cysteine, suggested a role in scavenging glutathione from the extracellular environment for the maintenance of sulphur homeostasis in this yeast.

583: Preliminary characterization of the IGF-II binding domain of the fugu fish mannose 6-phosphate/IGF-II receptor protein

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In mammals, it is well established that two mannose 6-phosphate receptor proteins designated as MPR's (MPR 300, Mr 300KDa also the IGF-II receptor and MPR 46, Mr 46KDa, cation dependent receptor). Our laboratory identified mammalian homologues of the receptors in different non-mammalian vertebrates and invertebrates. We further established that the mannose 6-phosphate binding regions of the receptors are conserved throughout the vertebrates from fish to mammals (fish: Xiphiphorus partial cDNA sequence, fugu fish: partial clones). Other workers found that the Zebrafish receptors also exhibit conserved mannose 6-phosphate binding domains. It is now clear that the mammalian MPR 300 and the chicken MPR 300 protein is also the receptor for the IGF-II and has distinct binding domains in its structure for the sugar and the IGF-II. Disruption of the negative regulatory effects of MPR 300 on IGF-II induced growth can lead to embryonic lethality and cancer promotion. In order to gain new insights into the IGF-II binding domain of the fish receptors, in the present study we cloned the 11th domain of the fugu fish MPR300 protein, using fugu cDNA as the template, the primers: F11S: GCGAATTCACCCAGCAGGACGA; F11AS: ACTCTAGATTTGC AGTCTGTTCGCAGAG. The amplified fragment ($\sim$500 bp) was sequenced and the sequence aligned by multiple sequence alignment with the known IGF-II domain sequences. We observed that in the fugu sequence, the critical isoleucine residue shown to be important in IGF-II binding in mammals is at a different position compared to the mammalian domain sequence. Of the AB loop, CD loop and FG loop regions seen in other IGF-II domain structures, the fugu sequence has only the two proline residues conserved in the FG loop as present in the known sequences. This is also seen in the chicken which has a leucine residue in place of the critical isoleucine. However, the chicken receptor can bind IGF-II. It would be interesting to see if the fugu receptor can also bind IGF-II, which is the future plan of work in our lab.

584: A Comparative analysis of the sulphur assimilatory pathways in *Candida glabrata* with other yeasts

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Sulphur is an important element required for the normal growth of the microorganism. Yeasts assimilate inorganic sulphur through a set of genes to convert them into sulphur containing amino acids like cysteine, methionine and the tripepetide glutathione. This complex network of genes for the assimilation of inorganic sulphur and their conversion to sulphur containing amino acids constitutes the sulphur assimilatory pathway. *Candida glabrata* is an emerging yeast pathogen which affects immunocompromised patients and now ranks second as a causative agent for Candidiasis after *Candida albicans*. A Relative comparison of the proteins involved in the sulphur assimilatory pathway of *Saccharomyces cerevisiae* was carried out against the *Candida glabrata* genome to identify the corresponding orthologus proteins in *Candida glabrata*. It was found that the sulphur assimilatory pathway was very similar to *Saccharomyces cerevisiae* in terms of inorganic sulphur utilization as well as the presence of the transsulfuration and reverse transsulfuration pathway, however, several unique aspects were observed in terms of organic sulphur utilization. To validate our observations regarding organic sulphur utilization in *Candida glabrata* we have created a disruption of the *met15* othologue of *Candida glabrata*. We have used this strain to evaluate different aspects of organic sulphur utilization in this yeast. This disrupting was an organic sulphur auxotroph and grew well on cysteine and methionine.

585: Gene identification signature (GIS) analysis for Zebrafish transcriptome characterization and genome annotation

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Rapid development, transparency and experimental accessibility of the embryo make the Zebrafish (Danio rerio) an important vertebrate model for biomedical research. Despite the almost completed Zebrafish draft genome sequencing, the comprehensive annotation of Zebrafish genome still lags behind. It is been recognized that comprehensive genome annotation with expressed genes and transcripts are fundamentally important to understand the zebrafish genome. Gene identification signature Paired-End diTag (GIS-PET) analysis characterizes the expressed full length cDNA transcript by extracting two ends for sequencing analysis. When mapping to the genome, GIS-PET accurately demarcates gene transcription boundaries, infer proximal promoter sites, enable the discovery of novel genes and alternative transcript variants. Using the GIS-PET approach, we comprehensively characterize Zebrafish transcriptome and identify both normal and unusual transcripts derived from Zebrafish genome. Here, we demonstrate this unique capability through the analysis of 5,898,233 unique PET which generate from five types of Zebrafish tissues. We have identified a large number of differentially expressed alternative 5′ and 3′ transcript variants, novel transcriptional units and hundreds fusion transcript candidates in different tissues here. In addition to the tag based approach, we have also collected over 100 K Zebrafish full length Genes through Colony Array Hybridization and EST sequencing in this study. Taking together, the comprehensive transcriptome characterization and full length cDNA clones offer valuable resources for the further Zebrafish and other biomedical research and the GIS-PET approach presented here promises to be a useful tool in annotating the vertebrate genomes.