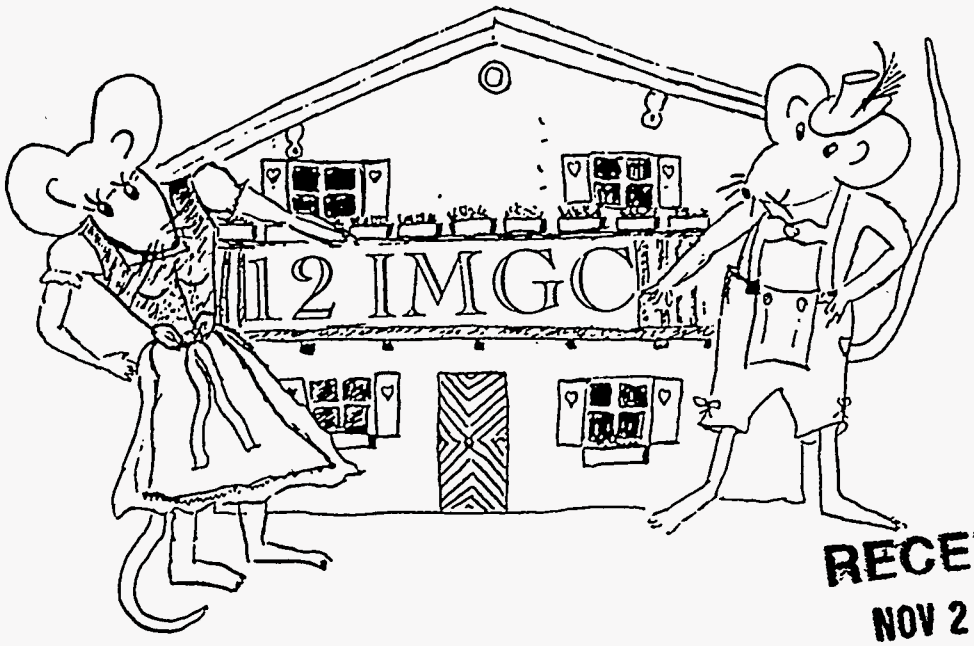

12th International Mouse Genome Conference

September 29 - October 03, 1998

Garmisch-Partenkirchen

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A MUTATION IN THE TOLL-4 GENE OF ENDOTOXIN-RESISTANT (C3H/HeJ) MICE IS RESPONSIBLE FOR THE DEFECT IN LPS SIGNAL TRANSDUCTION

Alexander Poltorak[1], Xiaolong He[2], Irina Smirnova[1], Mu-Ya Liu[3], Xin Du[1], Dale Birdwell[1], Erica Alejos[1], Maria Silva[1], Christophe Van Huffel[4], and Bruce Beutler[1]

ABSTRACT

Mutations of the *Lps* gene of mice are known to selectively impede LPS signal transduction, yielding a phenotype characterized by resistance to all biological effects of endotoxin and exaggerated susceptibility to overwhelming gram-negative infection. *Lps* was confined to an interval 0.9 cM in genetic size, based on 2093 meioses, produced by backcrosses of C3H/HeJ mice against animals of two separate Mus musculus strains (SWR and C57BL/6). A contig containing 63 BAC clones and 20 YAC clones was assembled to span this region. A large and irreducible "zero region" was observed wherein no crossovers were detected, and the physical distance between the limiting markers was found to be 2.6 Mb. A search for genes was undertaken, using exon trapping, hybridization/selection, and high-density genomic sequencing. In the course of this search, nearly 40,000 sequencing reads were examined by BLAST searches and by GRAIL analysis. Within the critical region, only two authentic genes were detected. A portion of the classical marker *Pappa* protruded into the critical region, and the mouse toll-4 receptor gene was represented in entirety. Toll-4 was considered an excellent candidate gene, based on the fact that the IL-1 receptor is a member of the toll superfamily, and on the additional fact that, in humans, a single mutation is known to abolish signal transduction through both the LPS and IL-1 pathways. We therefore searched for mutations of toll-4 in LPS-resistant mice of the strain C3H/HeJ. We identified a single nucleotide substitution, predicted to replace an evolutionarily conserved proline with a histidine residue in the cytoplasmic domain of the toll-4 protein. Control animals of the substrain C3H/HeN (believed nearly identical to C3H/HeJ) did not bear the mutation, nor did mice of four other *Mus musculus* strains. While the *Drosophila* toll protein is an important component of the drosomycin antifungal response pathway, we conclude that the mammalian toll-4 protein has been adapted primarily to the recognition of LPS. Destructive mutations of toll-4 predispose to the development of gram-negative sepsis, and do so in a highly specific fashion. Further, mutations of proteins that signal from toll-4 are likely to cause immunodeficiency.

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ACKNOWLEDGEMENTS

The meeting was funded in part by the National Human Genome Research Institute (Grant HG00756-06) and the US Department of Energy Office of Health and Environmental Research (Grant DE-FG02-98ER62634). Additional support was provided by the National Institute for Child Health and Human Development, National Cancer Institute and National Center for Research Resources of the National Institutes of Health. In addition, support was received from the Deutsche Forschungsgemeinschaft (DFG) and the Bayerisches Staatsministerium für Unterricht, Kultus, Wissenschaft und Kunst. Support was also received from the International Mammalian Genome Society and Mouse News Letter Ltd.

Assistance and support was received from the GSF in Neuherberg. Special acknowledgements to Health Reseach, Inc. of Roswell Park Cancer Institute, New York State Department of Health and the Roswell Park Cancer Institute Office of Scientific Communications for assistance in the preparation of the abstract booklet, management of workshop finances and general support services they have rendered.

We would like to specially acknowledge the help and great assistance of Claudia Erbelding and Darla Miller who organized the administrative part of the meeting. Special thanks also to Heidi Peczkowski and Änne Schubert for their assistance.

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PREFACE

This book contains abstracts of the platform and poster presentations of the Twelvth International Mouse Genome Conference, September 30- October 3, 1998, Garmisch-Partenkirchen, Germany.

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Thursday Oct. 1st

(A) Genetic and Physical Mapping — Chair: Jean-Louis Guenet

8:30-9:15	Nick Hastie	*Verne Chapman Lecture*: Dissecting a Human Contiguous Gene Syndrome with the Help of Mouse Models (A1)
9:15-9:30	Mike Seldin	Telomere Length Regulation in Mice is Linked to a Novel Chromosome Locus (A2)
9:30-9:45	Lorraine Eley	Transcript Mapping in the Mouse using Radiation Hybrids (A3)
9:45-10:00	Matthias Platzer	Comparative Sequence Analysis of 1.15 Mb of Genomic DNA from Mouse X Chromosome and the Corresponding Regions of Human Xq28 (A4)
10:00-10:15	Alan Charest	Pushing the Boundaries of Mouse Genetics: Towards the Development of a High Throughput Genotyping System in Closely Related *Mus. musculus* Strains (A5)
10:15-10:30	Lucy Rowe	Mouse Radiation Hybrid (RH) Mapping: Some Early Lessons we are Learning (A6)
10:30-10:45	Stephen Kingsmore	Construction of a High Resolution Radiation Hybrid Map of the Rat Genome (A7)

10:45-12:30 *Coffee* and **Posters on Mutagenesis (F)** — **Chair: Maja Bucan**

12:30-14:00 *Lunch* — *Chromosome Chairs Meeting*

(B) Gene Identification and Mutation Analysis — Chair: Miriam Meisler

14:00-14:15	David Beier	Analysis of Single-nucleotide Polymorphisms (SNPs) in the Mouse (B1)
14:15-14:30	Jack Favor	Loss of Heterozygosity at the *dilute - short ear* Region of the Mouse: Mitotic Recombination or Double Non-disjunction? (B2)
14:30-14:45	Colin Fletcher	*nervous* Encodes a Novel Protein with Limited Homology to Proteins Involved in Lipid Metabolism (B3)
14:45-15:00	Neal Copeland	The *itchy* Locus Encodes a Novel Ubiquitin Protein Ligase that is Disrupted in a18H Mice (B4)
15:00-15:15	Jennifer McKee-Johnson	Mutations in *Pmca2* Underly the deafwaddler and deafwaddler 2J Phenotypes (B5)
15:15-15:30	Jane Barclay	Positional Cloning of the Mouse Neurological Mutant ducky (*du*) (B6)
15:30-15:45	Murray Brilliant	A Very Large Novel Protein with Diverse Functional Motifs is Deficient in *rjs* (runty, jerky, sterile) Mice (B7)
15:45-16:00	Ulrike Teichmann	Molecular Analysis of Mouse Neural Crest Development and Disease using cDNA Expression Profile Analysis (B8)
16:00- 16:15	Velizar Tchernev	Identification of Proteins that Interact with the beige (Chediak-Higashi syndrome) Protein (LYST), using a Yeast Two-hybrid Method (B9)

16:15-18:00 *Coffee* and **General Posters**

18:00-20:00 *Dinner* — *Nomenclature Committee*

20:00-20:45 Oliver Smithies Essential Hypertension - A Genetic Disease Due to Many Little Things? (C1)

Friday Oct. 2nd

(C) Functional Genomics I — Chair: Paul Denny

8:30-9:15	Nabuyo Maeda	Genetic Disorders of Atherosclerosis in Mice (C2)
9:15-9:30	Catherine Nguyen	Using Knock-out Mice and Quantitative Expression Analysis to Address Immunological Questions (C3)
9:30-9:45	Piero Carninici	Highly Efficient Synthesis of Full-length cDNA by Trehalose Thermoactivated Reverse Transcriptase (C4)
9:45-10:00	Zdenek Trachtulec	Processing of Mouse Genes Encoded by a YAC in *S. cerevisiae* (C5)
10:00-10:15	Eugene Rinchik	The Chromosome-7 Mutagenesis Program at the Oak Ridge National Laboratory (C6)
10:15-10:30	Franz Vauti	Functional Analysis of Mammalian Genes by a Large Scale Gene Trap Screen in Mouse Embryonic Stem Cells (C7)
10:30-10:45	Kenichi Yamamura	Exchangable Gene Trap as a Tool for Random Mutagenesis (C8)
10:45-11:00	Gail Herman	The Mouse Bare Patches and Striated Gene Encodes a Putative, Novel 3β-hydroxysteroid Dehydrogenase (C9)

11:00-12:30 *Coffee* and **Posters on Bioinformatics (G)** — **Chair: Janan Eppig**

12:30-14:00 *Lunch* — *HUGO Mouse Committee*

Functional Genomics II — Chair: Wayne Frankel

14:00-14:15	Hee-Kyung Hong	A High Resolution Physical and Transcript Map of the satin - Congenital Hydrocephalus Region of Mouse Chromosome 13 (C10)
14:15-14:30	Christoph Kellendonk	Genetic Dissection of Glucocorticoid Receptor Function (C11)
14:30-14:45	Hee Sup Shin	Multiple Defects in Hippocampal Functions in Phospholipase -C-b1 Deficient Mice (C12)
14:45-15:00	Eirikur Steingrimmson	Genetic Analysis of the Microphthalmia Family of bHLHZip Transcription Factors (C13)
15:00-15:15	Roger Cox	ENU Induced Embryonic Lethal Mutations of the *quaking* gene: Contrasting Effects (C14)
15:15-15:30	Joe Nadeau	Cholesterol and the Hedgehog Signal Transduction Pathway: Evidence for an Interaction with Homocysteine Metabolism and Implications for Developmental Defects (C15)
15:30-15:45	Steve Brown	*XIST* Transgenes on the Mouse Y Chromosome Act as a Single Counting Centre *in vivo* (C16)
15:45-16:00	Yoshihide Hayashizaki	Full-length Mouse cDNA Analysis by Automated Fluorescent 384 Capillary Sequencer System (RISA: Riken Integrated Sequence Analysis System) (C17)
16:00-16:15	Werner Müller	Postnatally Induced Inactivation of *gp130* in Mice Results in Neurological, Cardiac, Hematopoietic, Immunological, Hepatic and Pulmonary Defects (C18)

16:15-18:00 *Coffee* and **General Posters** — *Chromosome Committee Meetings*

18:00-20:00 *Dinner* — *IMGS Secretariat Meeting*

Saturday Oct 3rd

(D) Quantative and Multigenic Traits — Chair: Joe Nadeau

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8:45-9:00	Tom van Wezel	Colon Cancer Susceptibility Genes (D2)
9:00-9:15	Simon Foote	Mapping Disease Modifier Loci for Malaria and Leishmania (D3)
9:15-9:30	Hiroshi Ikegami	Marker-assisted Congenic Strategy in Combination with Historical Recombinants for Pinpointing Candidate Genes for Complex Traits: Application for an Insulin-dependent Diabetes Susceptibility Gene (*Idd3*) of the Nonobese Diabetic (NOD) Mouse (D4)
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9:45-10:00	Karen Moore	The Genetic Dissection of Low IgE Response in the SJL/J Mouse Inbred Strain (D6)

10:00-10:30 **Discussion on QTLs** — **Chair: Karen Moore**

10:30-12:30 *Coffee* and **General Posters**

12:30-14:00 *Lunch* — *Editorial Board Meeting Mammalian Genome*

(E) Genetics of Development & Differentiation — Chair: Kenichi Yamamura

14:00-14:45	Pascal Haffter	From Phenotype to Gene: Mapping of Mutations and Genes in the Zebrafish (E1)
14:45-15:00	Michael Reed	An *H19* Deletion in the Mouse Randomly Destabilizes Genomic Imprinting of the Coregulated *H19* and *Igf2* Genes (E2)
15:00-15:15	Antonis Hatzopoulos	Endothelial Embryonic Progenitor Cells as a Model to Study Cardiovascular Development and Diseases (E3)
15:15-15:30	Mary Wines	Identifying Candidate Genes and New Phenotypes for the Proximal Albino Deletions (E4)
15:30-15:45	Yuichi Oike	Disruption of the *CBP* Gene Affects Facial and Cardiac Development, Long-Term Memory and Early Haematopoiesis (E5)
15:45-16:00	Kurt Wünsch	Maintenance of Early CNS And PNS Structures Requires the Delta Homologue Dll1 (E6)

16:00-18:00 *Coffee* and ***IMGS Business Meeting***

18:00 *Bus to Dinner*

19:00- 21:00 *Bavarian Evening*

A. Genetic and Physical Mapping

Abstracts - Presentations

A1. The Verne Chapman Memorial Lecture

DISSECTING A HUMAN CONTIGUOUS GENE SYNDROME WITH THE HELP OF MOUSE MODELS.

Nicholas D. Hastie, *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh.*

A2. TELOMERE LENGTH REGULATION IN MICE IS LINKED TO A NOVEL CHROMOSOME LOCUS.

M.F. Seldin[1], *L. Zhu*[1], *K.S. Hathcock*[2], *P. Hande*[3], *P.M. Lansdorp*[4], *R.J. Hodes*[2]. [1]*Rowe Program in Genetics, Univ. of California, Davis, Davis CA;* [2]*National Institute on Aging, Nat. Inst. of Health, Bethesda, MD;* [3]*Terry Fox Lab. For Hem./Onc., BC Cancer Research Center, Vancouver, BC.*

A3. TRANSCRIPT MAPPING IN THE MOUSE USING RADIATION HYBRIDS.

Paris Group: *Philip Avner*[1,2], *Isabelle Poras*[1], *Gabor Gyapay*[1], *Cecile Fizames*[1], *Nunchanard Chianniluchai*[1], *William Saurin*[1], *Jean Weissenbach*[1]. [1]*Genescope-Centre National de Sequencage, 2 rue Gaston Cremieux, 91006 Evry, France;* [2]*Unite de Genetique Moleculaire Murine, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France.*
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London Group: *Rosa Beddington and Sally Dunwoodie, Laboratory of Mammalian Development, National Institute for Medical Research, London, Mill Hill, UK.*
Cambridge Group: *Patricia Rodriguez-Tome, Jeremy D. Parsons: European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK.*

A4. COMPARATIVE SEQUENCE ANALYSIS OF 1.15 Mb OF GENOMIC DNA FROM MOUSE X CHROMOSOME AND THE CORRESPONDING REGIONS OF HUMAN Xq28.

Platzer, M[1], *Wiehe, T*[1], *Reichwald, K*[1], *Nordsiek, G*[1], *Zhao, W*[2], *Herman, GE*[2], *Rosenthal, A*[1,3]. [1]*Institute of Molecular Biotechnology, Jena, Germany;* [2]*Ohio State University, Columbus, Ohio, US;* [3]*Friedrich-Schiller Univerisity, Jena, Germany.*

A5. PUSHING THE BOUNDARIES OF MOUSE GENETICS: TOWARDS THE DEVELOPMENT OF A HIGH THROUGHPUT GENOTYPING SYSTEM IN CLOSELY RELATED MUS. MUSCULUS STRAINS.

Alan Charest, *Viktoria Kheifets, John E. Landers, Barbara M. Jordan and David E. Housman. Center for Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA, 02139.*

A6. MOUSE RADIATION HYBRID (RH) MAPPING: SOME EARLY LESSONS WE ARE LEARNING.

Lucy Rowe, *Mary Barter, Patricia Rodriguez-Tomé, Juergen Naggert, Janan Eppig.*

A7. CONSTRUCTION OF A HIGH RESOLUTION RADIATION HYBRID MAP OF THE RAT GENOME.

CC Glenn[1], *AE Kwitek-Black*[2], *SD Colman*[1], *RM Peitzsch*[1], *D Marks*[1], *MP Popp*[1], *M Granados*[2], *T Mull*[2], *M Kissebah*[2], *J Lu*[2], *MC Peden*[1], *C Griswold*[1], *P Duelly*[1], *J Hudson*[3], *L Stein*[4], *HJ Jacob*[2], **SF Kingsmore**[1]. [1]*CuraGen Corporation,* [2]*Medical College of Wisconsin,* [3]*Research Genetics, Inc.,* [4]*Cold Spring Harbor Laboratory.*

A. Genetic and Physical Mapping

A1. The Verne Chapman Memorial Lecture

DISSECTING A HUMAN CONTIGUOUS GENE SYNDROME WITH THE HELP OF MOUSE MODELS.

Nicholas D Hastie, *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh.*

Human chromosome deletions have been a powerful resource for the identification of genes associated with congenital disorders, particularly developmental disorders. Genes identified through this route have been shown to play pivotal roles in normal vertebrate development. For the past decade or so we have been studying the human contiguous gene syndrome, WAGR. Children with this syndrome have constitutional hemizygous deletions on the short arm of chromosome 11 (11p13) and have characteristic disorders, including Wilms' tumour of the kidney, Aniridia (lack of an iris), Genitourinary abnormalities and mental Retardation. Through positional cloning 2 genes in the deletion region have been identified which, when mutated, lead to at least 3 of these conditions. Mutations in the Wilms' tumour suppressor gene, WT1, can lead to both the eponymous tumour and genitourinary abnormalities and mutations in the PAX6 gene lead to aniridia. No particular gene has been associated with mental retardation as yet.

The PAX6 gene encodes a protein that has a paired-type homeobox as well as a more conventional homeobox. This protein is highly likely to encode a transcription factor. The WT1 gene encodes a protein with 4 zinc fingers that also has all the hallmarks of a transcription factor. However, our own recent evidence suggests that WT1 may function both in transcription and splicing and that different, alternative isoforms of WT1 may have different functions.

I will describe how different mutations in these 2 genes in humans have helped us to dissect function. However, to unravel the function of these genes we must turn to the mouse as a model system. Studies in homozygous Smalleye mutant mice have shown that PAX6 is required for the normal development of the eye, nose and central nervous system. Heterozygous Smalleye mice have a small eye but also suffer from aniridia and hence offer an excellent model for the human condition. Mouse knockouts have shown that wt1 is essential for the formation of the genitourinary system and mesothelium.

As one approach to understanding the function and regulation of these genes we have been using YAC transgenesis to introduce wildtype and modified genes into mice to study expression and gene function. These experiments have given new insights into gene dosage requirements, long distance regulators and developmental function.

There appears to be high conservation of synteny between a region of mouse chromosome 2 and the human 11p13 region. Several hemizygous deletions have been described in this region which remove the PAX6 gene, resulting in the Smalleye phenotype. Using these deletions we are intending to saturate this region for recessive mutations using ENU mutagenesis in collaboration with Steve Brown and Jo Peters at Harwell. This, in turn, should help us to discover novel genes associated with human abnormalities. To help identify genes in the region we have cloned the corresponding region from the puffer fish, fugu rupripes; this is proving to be a very productive route. To explore gene function and evolution we are introducing fugu genes into mice by transgenic approaches.

A2. TELOMERE LENGTH REGULATION IN MICE IS LINKED TO A NOVEL CHROMOSOME LOCUS.

M.F. Seldin[1], *L. Zhu[1], K.S. Hathcock[2], P. Hande[3], P.M. Lansdorp[4], R.J. Hodes[2]. [1]Rowe Program in Genetics, Univ. of California, Davis, Davis CA; [2]National Institute on Aging, Nat. Inst. of Health, Bethesda, MD; [3]Terry Fox Lab. For Hem./Onc., BC Cancer Research Center, Vancouver, BC.*

Telomeres are DNA-protein complexes at chromosomal termini which are critical to maintaining chromosomal integrity and have been implicated in regulation of cellular replicative capacity. Telomere length is the net resultant of the loss of telomeric repeats that occurs with chromosomal replication, and of compensatory mechanisms, such as that mediated by telomerase, which are capable of extending telomere length. To investigate the genetic regulation of telomere length, an analysis was carried out using two species of mice which differ substantially in their telomere length. *Mus musculus domesticus* (telomere length >25 kb) and *Mus spretus* (Spain) (telomere length 5-15 kb) were used to generate F1 crosses and reciprocal backcrosses, which were then analyzed for regulation of telomere length. This analysis indicated that a dominant and trans-acting mechanism exists capable of extensive elongation of telomeres in somatic cells following fusion of parental germline cells with discrepant telomere lengths. A genome wide screen of interspecific crosses, using Mus spretus as the recurrent parent, identified a region on distal chromosome 2 that predominantly controls the observed species-specific telomere length regulation. Each of 54 short telomere backcross mice were homozygous for the *M. spretus* genotype at *D2Mit74*; and all of the 90 mice that had the heterozygous genotype at this locus (SB genotype) had the long telomere phenotype. Considering only the 54 short telomere phenotype mice, the chances of observing only the homozygous spretus genotype at this locus in a genome wide analysis is $<< 10^{-10}$. The observation that 18 of 72 mice that were homozygous for the Mus spretus genotype at this locus had the long telomere phenotype indicates that this locus cannot account for all of the genetic control of telomere length in these crosses. Additional microsatellites on distal mouse chromosome 2 were examined in the short telomere phenotype mice to more precisely define the critical genetic interval. These studies indicate that the putative gene for this phenomenon is located in the distal 5 cM of mouse chromosome 2. This locus is distinct from candidate genes encoding known telomere-binding proteins or telomerase components which were mapped to disparate chromosomal locations: *Tep1*, 18.9 cM on Chr 14; *Terf1*, 11.8 on Chr 1; *Terf2*, 50 cM on Chr 8; and Tert, 49 cM on Chr13. These results demonstrate that a novel unidentified gene on distal mouse chromosome 2 can regulate telomere length in the mouse. No apparent candidate genes or ESTs have been identified in the mapped interval of mouse chromosome 2 or in the homologous region of the long arm of human chromosome 20 (20q13.2-13.3). Additional crosses are in progress to further narrow the interval for positional cloning of the putative gene responsible for determination of species-specific telomere length. This approach may have substantial implications with respect to understanding telomere length regulation in mammalian cells and conditions including malignancy and aging, in which such function may be important in determining tumor cell survival or longevity.

A3. TRANSCRIPT MAPPING IN THE MOUSE USING RADIATION HYBRIDS.

Paris Group: *Philip Avner[1,2], Isabelle Poras[1], Gabor Gyapay[1], Cecile Fizames[1], Nunchanard Chiannilukai[1], William Saurin[1], Jean Weissenbach[1]. [1]Genescope-Centre National de Sequencage, 2 rue Gaston Cremieux, 91006 Evry, France; [2]Unite de Genetique Moleculaire Murine, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France.*
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London Group: *Rosa Beddington and Sally Dunwoodie, Laboratory of Mammalian Development, National Institute for Medical Research, London, Mill Hill, UK.*
Cambridge Group: *Patricia Rodriguez-Tome, Jeremy D. Parsons: European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK.*

An EU funded consortium co-ordinated through the Paris laboratory is constructing a transcript map of the mouse genome using the radiation hybrid panel T31 constructed by Linda McCarthy (Genome Research 7:1153-1161, 1997) and distributed through Research Genetics. The aim of the consortium is to generate a robust framework map and to map 10,000 ESTs. The ESTs are being obtained both from public databases and from sequence generated from the London laboratory embryo libraries. Pancreatic islet libraries are being generated in Oxford from late embryos over the time course of islet formation, and will be incorporated in a programme of sequencing and EST generation later in the project. Large scale sequencing of ESTs is being carried out in the Berlin laboratory. Cambridge have created an EST clustering, allocation and browsing workbench to assist International collaboration and minimise wasted STS mapping effort. Using public CORBA servers, and Java applets, a user can browse from EST clusters down to traces and sequences or up to the Radiation Hybrid Database (see Parsons and Rodriguez-Tome abstract). We will discuss the consortium plans to offer a mapping service through the Milano laboratory. And we will describe the current status of the framework marker maps, of EST generation and mapping, and of the library construction experiments.

A4. COMPARATIVE SEQUENCE ANALYSIS OF 1.15 Mb OF GENOMIC DNA FROM MOUSE X CHROMOSOME AND THE CORRESPONDING REGIONS OF HUMAN Xq28.

Platzer, M[1], *Wiehe, T[1], Reichwald, K[1], Nordsiek, G[1], Zhao, W[2], Herman, GE[2], Rosenthal, A[1,3]. [1]Institute of Molecular Biotechnology, Jena, Germany; [2]Ohio State University, Columbus, Ohio, US; [3]Friedrich-Schiller Univerisity, Jena, Germany.*

The distal end of the long arm of the human X-chromosome, Xq28, is one of the most gene dense regions of the human genome. Also, many disease genes have been mapped to this region. Sequencing of Xq28 is well underway and at present more than 4 Mb have been completed, most of it by the Genome Sequencing Center in Jena. The available sequence data reveals a striking heterogeneity in this cytogenetic band as far as gene density, repeat content and base composition are concerned.

In order to better understand the organization and evolution of this human chromosomal band we have started comparative sequencing of syntenic regions of the Mouse X chromosome, band A. This approach will enable us to find evolutionarily conserved regions between mouse and man. These regions will be investigated for their coding or regulatory potential. In particular, we are interested to find evidence for new genes or regulatory elements within large introns or intergenic regions, differential splicing and evolutionary breakpoints or re-arrangements.

To built larger contigs around mouse markers *DXMit120, DXMit25,* FALE8, *Trap* and *Mecp2* we have isolated and sequenced five BACs (http://genome.imb-jena.de/printMouse.html). PCR products from the ends of these seed clones were then used to isolate overlapping clones. Following this mapping strategy we have constructed and sequenced several contigs comprising 1.15 Mb of mouse genomic DNA including the genes of *Mtm1, Mtmr1, Aldgh, Mssk1, Idh, Trap, L1cam, Avpr2, C1, Te2, Rbp, Hcfc1, Irak, Mecp2* and the 5'part of the color vision locus.

We will present a detailed analysis of mouse and human sequence data using pairwise alignments based on the SIM algorithm as well as complex tools like RUMMAGE for repeat and gene identification. For selected coding and non coding regions we compare their local rates ofdivergence.

This work contributes to the systematic comparative analysis of mouse X chromosome within 4 cM between *Ids* and *Dmd* (http://www.mgc.har.mrc.ac.uk/comp_seq; see also P. Denny et al. at this conference).

A5. PUSHING THE BOUNDARIES OF MOUSE GENETICS: TOWARDS THE DEVELOPMENT OF A HIGH THROUGHPUT GENOTYPING SYSTEM IN CLOSELY RELATED MUS. MUSCULUS STRAINS.

Alan Charest, *Viktoria Kheifets, John E. Landers, Barbara M. Jordan and David E. Housman. Center for Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA, 02139.*

The current methods of genotyping inbred strains of mice depend heavily on costly and time consuming manipulations. The goal of this study is to dramatically reduce these constraints. We have devised a genotyping approach which relies on two principles: 1) a reduction in the complexity of the genome by generating short Interspersed Repetitive Sequence (IRS)-PCR DNA fragments which represent the whole genome and 2) the identification of sequence differences within these fragments even among closely related strains. As an initial step towards the implementation of this strategy, the frequency of polymorphism amongst many inbred strains in several regions surrounding and including B1 repeats was assessed by SSCP. We found that the rate of polymorphism varies from 30% to 85% which relates to the phylogenecity of the strains under analysis. These data indicate that Single Nucleotide Polymorphisms (SNPs) in inter-B1 regions between closely related inbred strains occur at a high frequency. The robustness and consistency of the IRS-PCR reaction allows for a large number of genotypings at loci throughout the genome to be carried out from a single PCR reaction. We are currently developing a series of strain-specific SNP markers from IRS-PCR fragments on the basis of these findings. These markers will be used in conjunction with a high throughput genotyping method based on Allele Specific Oligonucleotide (ASO) hybridization. This highly efficient and low cost genotyping system will invariably facilitate the mapping of modifier loci and QTLs between closely related inbred strains of the Mus. Musculus genus.

A6. MOUSE RADIATION HYBRID (RH) MAPPING: SOME EARLY LESSONS WE ARE LEARNING.

Lucy Rowe, *Mary Barter, Patricia Rodriguez-Tomé, Juergen Naggert, Janan Eppig.*

In collaboration with EBI's RHdb, The Jackson Laboratory Mapping Panel Resource is now maintaining a comprehensive database for the mouse T31 Radiation Hybrid mapping data. This new database operates in a similar manner to the Jackson Laboratory Backcross database by offering detailed analysis of newly submitted RH mapping data, with technical support, and WWW posting of data and maps. All public data are exchanged between RHdb at EBI and The Jackson Laboratory RH database at regular intervals. In June 1998 there were over 500 loci placed in this public RH database, from 14 laboratories. Most chromosomes are not yet densely enough mapped to achieve significant linkage along their entire length. However, most new data sets do find significant match to one or more previously mapped loci.

Typing the RH panel has provided unexpected technical challenges. Robust mouse-specific signals are often accompanied in other cell lines by weak ambiguous bands, duplicate assays are not always in complete concordance, some hamster bands interfere with clean scoring, results from different laboratories for the same marker are often slightly different, some markers appear to have artifactually high or low retention frequencies, many SSLP markers give allele differences that make scoring complicated, a given cell line may have many fragments from a single chromosome making precise ordering of markers uncertain, and inserting new loci into the map most often expands the distances between flanking markers. For some mappers, these kinds of problems may stand in the way of successful mapping with this system. For others, the increase in resolution over recombination mapping has proven to be very useful.

Successful users of the T31 panel are generally mapping several closely linked markers in a region, using highly sensitive assays, screening the panel in duplicate for each marker, considering repeatable weak signals as positive, and anchoring their RH maps to the recombination based map at frequent intervals. These methods take advantage of the strength of the RH panels: their higher resolution for mapping over short intervals, or in separating and ordering loci that fail to recombine in conventional linkage crosses.

A7. CONSTRUCTION OF A HIGH RESOLUTION RADIATION HYBRID MAP OF THE RAT GENOME.

CC Glenn[1], AE Kwitek-Black[2], SD Colman[1], RM Peitzsch[1], D Marks[1], MP Popp[1], M Granados[2], T Mull[2], M Kissebah[2], J Lu[2], MC Peden[1], C Griswold[1], P Duelly[1], J Hudson[3], L Stein[4], HJ Jacob[2], **SF Kingsmore[1].** *[1]CuraGen Corporation, [2]Medical College of Wisconsin, [3]Research Genetics, Inc., [4]Cold Spring Harbor Laboratory.*

The use of radiation hybrids (RH) and RH maps has revolutionized physical mapping of disease loci in human. The elegance of this approach is in its simplicity; a single 96-well PCR reaction can map a candidate gene with relatively high resolution. Until now, there has not been an RH map for the rat and thus, there has been limited molecular access to traits studied in rat models. The construction of a rat RH map is of great significance because the rat has been, and continues to be utilized as a powerful model system for investigating human genetic diseases, particularly multifactorial diseases, physiological traits and in drug toxicology screening.

We have used a high throughput, PCR-based method to construct a completely closed radiation hybrid map of the rat genome using the rat whole genome radiation hybrid panel generated in Peter Goodfellow's laboratory (available from Research Genetics, Inc.). This newly constructed radiation hybrid map was assembled using the RHMAPPER software package. This map, which includes a subset of the markers used to construct the Massachusetts General Hospital/Harvard Medical School, Whitehead Institute/MIT, and Medical College of Wisconsin rat genetic map, provides the opportunity to rapidly position candidate genes with respect to genetic loci and traits that have been mapped genetically. The map consists of a LOD 3 framework of 436 markers, plus an additional 1320 markers placed at lower likelihoods relative to the framework map, providing an average marker spacing of 3.9 cM for the framework and 1.3 cM overall. The spacing will be reduced as more markers are incorporated into the framework. By comparison with the genetic map, we estimate the coverage to be greater than 98% of the rat genome. This map will be publicly available at HYPERLINK http://www.curagen.com www.curagen.com and HYPERLINK "http://legba.ifrc.mcw.edu" http://legba.ifrc.mcw.edu after the framework is fully validated. In addition, CuraGen Corporation and the Medical College of Wisconsin plan to provide a free service for academic laboratories to map rat genes through these sites.

A. Genetic and Physical Mapping

Abstracts - Posters

A8. **A NEW MEMBER OF THE P53 FAMILY: MOLECULAR CLONING AND CHROMOSOMAL MAPPING OF THE GENE CODING FOR THE HUMAN TRANSCRIPTION FACTOR KET AND ITS MURINE HOMOLOG.**

Martin Augustin[1], *Casimir Bamberger*[2], *Dieter Paul*[1], *Hartwig Schmale*[2]. [1]*Fraunhofer Institute of Toxicology and Aerosol Research, Center for Medical Biotechnology, D-30625 Hannover, Germany, Tel: +49 511 5350 520, FAX: +49 511 5350 155; E-Mail: augustin@ita.fhg.de.* [2]*Institute of Cell Biochemistry and Clinical Neurobiology, University Hospital Hamburg-Eppendorf, D-20246 Hamburg, Germany.*

A9. **A NOVEL MUTATION SHABBY (*Shby*) MAPS TO CHROMOSOME 17.**

Simon T. Ball, *Terry M. Hacker, Edward P. Evans, Jo Peters. Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxfordshire, OX11 0RD, UK.*

A10. **THE JACKSON LABORATORY MAPPING PANELS: BACKCROSS AND RADIATION HYBRID DATABASES.**

Mary Barter, *Lucy Rowe, Michael Rourk, Juergen Naggert, Janan Eppig.*

A11. **SEQUENCING THE MOUSE Y CHROMOSOME SHORT ARM.**

H. Boettger-Tong[1], *W.J. Huang*[1,2], *A. Agoulnik*[1], *T. Ty*[1], *W. Harrison*[4], *R. Gibbs*[5], **C.E. Bishop**[1,3]. *Department of Obstetrics and Gynecology*[1]*; Scott Department of Urology*[2]*; Department of Molecular and Human Genetics*[3]*; Genome Sequencing Center*[5]*; Baylor College of Medicine, Houston TX; Department of Pathology, UT Medical School*[4]*, Houston TX.*

A12. **SEX-LINKED FIDGET, A MUTATION WHICH AFFECTS COCHLEAR DEVELOPMENT, IS ASSOCIATED WITH RECOMBINATION SUPPRESSION IN THE *DXMit109-Pou3f4* REGION.**

Yvonne Boyd, *Vivienne Reed, Graham Fisher, Walter Masson. MRC Mammalian Genetics Unit, Harwell, Oxon OX11 0RD, UK.*

A13. **GENETIC AND PHYSICAL CHARACTERIZATION OF THE REGION AROUND *waltzer* ON MOUSE CHROMOSOME 10.**

Elizabeth C. Bryda. *Department of Microbiology, Immunology & Molecular Genetics, Marshall University School of Medicine, Huntington,WV, USA.*

A14. **AMES waltzer (av): PHYSIOLOGICAL AND HISTOLOGICAL CHARACTERIZATION AND GENETIC MAPPING TO MOUSE CHROMOSOME 10 AT THE BORDER OF 10q21 AND 22q11 SYNTENY.**

M. Burmeister[1,2,3], *Y. Raphael*[4], *D. Sufalko*[1], *E. Zobeley*[1], *D. Dolan*[4], *K. Kobayashi*[4], *J. Henderson*[4], *S. Adkins*[1,2]. [1]*Mental Health Research Institute,* [2]*Dept. of Human Genetics,* [3]*Dept. of Psychiatry,* [4]*Kresge Hearing Research Institute, University of Michigan, Ann Arbor, USA.*

A15. **COMPARATIVE AND PHYSICAL MAPPING OF CONSERVED LINKAGE REGIONS WITH HSA21 AND IMPLICATIONS FOR DS MODELS.**

T. Wiltshire, **D.E. Cabin**, *S.E. Cole, M. Villanueva, R.H. Reeves. Dept. of Physiology, Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.*

A16. **PHYSICAL MAPPING WITHIN THE UROGENITAL SYNDROME REGION ON PROXIMAL MMU2.**

Deanna M. Church, *David LePage*[1], *Ron Conlon*[1], *Janet Rossant. Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Ontario.* [1]*Department of Genetics, Case Western Reserve, Cleveland, Ohio.*

A17. **AN 85KB TANDEM TRIPLICATION IN THE SLOW WALLERIAN DEGENERATION (*Wld*^{2}) MOUSE.**

Michael P. Coleman, *Laura Conforti, E. Anne Buckmaster, Andrea Tarlton, Robert M. Ewing, Michael C. Brown, Mary F. Lyon, V. Hugh Perry. Department of Pharmacology, University of Oxford, U.K. MRC Mammalian Genetics Unit, Harwell, U.K.*

A18. **CHROMOSOMES IN THE BARE PATCHES AND STRIATED CRITICAL REGION.**

Denny P[1], *Mallon A-M*[1], *Bates R*[1], *Strivens MA*[1], *Brown SDM*[1], *Botcherby MRM*[2], *Straw R*[2], *Fernando S*[2], *Williams GW*[2], *Weston P*[2], *Gardner P*[2], *Woollard PW*[2], *Gilbert M*[2], *Goodall K*[2], *Greystrong JS*[2], *Clarke D*[2], *Mundy, CR*[2], *Rhodes M*[2], *Dangel A*[3], *Cunningham D*[3], *Herman GE*[3], *Miller W*[4], *Platzer M*[5], *Wiehe T*[5], *Nordsiek G*[5], *Rosenthal A*[5]. [1]*MRC UK Mouse Genome Centre and Mammalian Genetics Unit, Harwell, Oxfordshire, UK.* [2]*MRC Human Genome Mapping Project Resource Centre, Hinxton, Cambridge, UK.* [3]*Department of Pediatrics and Children's Hospital Research Foundation, Ohio State University, Columbus, Ohio 43205, USA.* [4]*Department of Computer Science, The Pennsylvania State University, University Park PA 16802, USA.* [5]*I Institut für Molekulare Biotechnologie, Beutenbergstraße 11, D-07745 Jena, Germany.*

A19. SCURFY: A SEVERE X-LINKED IMMUNE DISEASE IN THE MOUSE.

Jonathan M.J. Derry, *Gary D. Means, Cindy R. Willis*, Dean Y. Toy, JoAnn C.L. Schuh*, Peter R. Baum. Departments of Molecular Biology and Molecular Immunology*, Immunex Corporation, 51 University St, Seattle WA 98101, USA.*

A20. PHYSICAL MAPPING OF THE REGION ON MOUSE CHROMOSOME 7 HOMOLOGOUS TO HUMAN CHROMOSOME 15q11-q13.

Madhu Dhar[1], *Lisa Webb*[1], *Barbara York*[3], *David West*[3], *Dabney Johnson*[1,2]. [1]*University of Tennessee Graduate School of Biomedical Sciences;* [2]*Life Sciences Division; Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077, USA;* [3]*Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124, USA.*

A21. NEW REARRANGEMENT BETWEEN MOUSE AND HUMAN X CHROMO-SOMES: TBL1 MAPS AT THE INTERFACE BETWEEN TWO LARGE REGIONS OF CONSERVATION.

Christine M. Disteche[1], *Mary Beth Dinulos*[2], *Maria Teresa Bassi*[2], *Rosemary W. Elliott*[3], *Elena I. Rugarli*[2]. [1]*Department of Pathology, BOX 357470, University of Washington, Seattle, Washington 98195-7470, USA;* [2]*Telethon Institute of Genetics and Medicine (TIGEM), San Raffaele Biomedical Science Park, Milano 20132, Italy;* [3]*Department of Molecular and Cell Biology, Roswell Park Cancer Institute, Buffalo, New York 14263, USA.*

A22. THE *fused phalanges* ALLELE OF *shaker-with-syndactylism* MAPS TO MIDDLE-DISTAL MOUSE CHROMOSOME 18, AND SPANS A REGION CONTAINING GENES SYNTENIC WITH HUMAN CHROMOSOME 5q.

J. Gazzard[1], *M. J. Dixon*[2], *K. P. Steel*[1]. [1]*MRC Institute of Hearing Research, University Park, Nottingham. United Kingdom. NG7 2RD.*[2] *School of Biological Sciences and Departments of Dental Medicine and Surgery, 3.239, Stopford Building, University of Manchester, Oxford Road, Manchester. United Kingdom. M13 9PT.*

A23. FINER MAPPING OF THE MOUSE MUTATION CALLED DOE WITH NEUROLOGICAL, EYE, AND COAT COLOR DEFECTS.

Mary Guarnieri[1,2], *Stacy Satornino*[3], *Jeffry Montgomery*[4], *Lorraine Flaherty*[1,2]. [1]*Molecular Genetics Program, Wadsworth Center, Albany, NY;* [2]*School of Public Health, SUNY Albany;* [3]*Department of Biological Sciences, SUNY Albany;* [4]*Kimmel Cancer Institute, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA.*

A24. MAPPING OF A MOUSE MOTOR NEURON DISEASE GENE, LOA, TO THE DISTAL REGION OF MMU12.

Nicholson S.J.[1]*; Witherden, A.S.*[1]*; Peters, J.*[2]*; Ball, S.T.*[2]*; Martin, J.E.*[3]*; Fisher, E.M.C.*[1]*;* **Hafezparast, M.**[1] [1]*Department of Neurogenetics, Imperial College School of Medicine at St. Mary's, London W2 1PG, UK;* [2]*Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxon, OX11 ORD, UK;* [3]*Department of Morbid Anatomy, The Royal London Hospital, Whitechapel, London, E1 1BB, UK.*

A25. MOUSE CHROMOSOME 4: A "HOT CHROMOSOME" FOR THE LOCATION OF TUMOR SUPPRESSOR GENES.

Herranz, M., *Meléndez, B., Pérez de Castro, I., Fernández, M., Reyes, J., Santos, J., Fernández-Piqueras, J. Departamento de Biología. Laboratorio de Genética Molecular Humana. Facultad de Ciencias. Universidad Autónoma de Madrid. 28049- Madrid. SPAIN.*

A26. RADIATION HYBRID MAPPING OF MOUSE EXPRESSED SEQUENCE TAGS (ESTs).

C. Heuston[1], *P.J. Trickett*[1], *A. Southwell*[1], *S. Greenaway*[1], *M.A.Strivens*[1], *T. Matise*[2], *A.R. Haynes*[1], *H. Doi*[3], *J. Kitchen*[3], *M. Ko*[3], *S.D.M. Brown*[1], *P.Denny*[1]. [1]*MRC UK Mouse Genome Centre and Mammalian Genetics Unit, Harwell, OX11 ORD, UK.* [2]*The Rockefeller University, 1230 York Ave, Box 192, New York, NY, USA.* [3]*Doi Bioasymmetry Project, ERATO, JST, Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI 48202, USA.*

A27. HYBRIDIZATION-BASED APPROACHES FOR INTEGRATED MOUSE GENOME MAPPING.

Heinz Himmelbauer[1], *Leonard C. Schalkwyk*[1], *Christina Nock*[1,2], *Ilona Dunkel*[1], *Stephanie Palczewski*[1], *Sabine Scheel*[1], *Gunther Wenske*[1], *Thomas Wissel*[1], *Markus Kramer*[1], *Joachim Klose*[2], *Hans Lehrach*[1]. [1]*Max-Planck-Institute for Molecular Genetics, Ihnestr. 73, D-14195 Berlin, Germany.* [2]*Institute for Human Genetics, Klinikum Rudolf Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany.*

A28. **MAPPING THE Hsc70, Hsc74, AND Hsp70RY MEMBERS OF THE MOUSE HEAT SHOCK PROTEIN FAMILY AND AN APPARENT LACK OF MOUSE HOMOLOGUES TO THE HUMAN Hsp70 HSPA6/7 GENES.**

Clayton R. Hunt[1], *Azemat J. Parsian*[1], *Robert J. Malyapa*[1], *Prabhat C. Goswami*[1], *Michael S. Watson*[2], *Christine A. Kozak*[3]. [1]*Dept. of Radiation Oncology,* [2]*Dept. of Genetics and Pediatrics, Washington University School of Medicine, St. Louis MO 62108;* [3]*National Institute of Allergy and Infectious Diseases, Bethesda MD 20892.*

A29. **CHARACTERIZATION AND GENETIC LOCALIZATION OF *strigosus (stg)* and *longjohn (lgj)*, NEW MUTATIONS AFFECTING GROWTH AND BODY SHAPE.**

Jean Jaubert[1], *Eva M. Eicher*[2], *Linda L. Washburn*[2], *Jean-Louis Guénet*[1]. [1]*Unité de Génétique des Mammifères de l'Institut Pasteur, 25-28 Rue du Docteur Roux, 75724 Paris Cedex 15, FRANCE.* [2]*The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609-1500, USA*

A30. **GENE CONTENT IN A FULLY-SEQUENCED 175 kb BAC CLONE FROM THE t-COMPLEX ON CHROMOSOME 17: 11 GENES INCLUDING *Nbps*, *Als* AND *D17Wsu15e*.**

George Kargul[1], *Ramaiah Nagaraja*[1], *Ellson Chen*[2], *Chun-Nan Chen*[2], *Peter Ma*[2], *Paul Waeltz*[1], *David Schlessinger*[1], *Minoru S.H. Ko*[1]. [1]*NIH/National Institute on Aging, Baltimore, MD 21224,* [2]*PE-Applied Biosystems, Foster City, CA 94404.*

A31. **GENOME ANALYSIS OF THE DISTAL IMPRINTED REGION OF MOUSE CHROMOSOME 7: COMPARATIVE SEQUENCING BETWEEN HUMAN AND MOUSE IDENTIFIES MULTIPLE TISSUE-SPECIFIC ENHANCERS IN THE DOWNSTREAM REGION OF H19.**

R. Kato[1], *K. Ishihara1, T. Yokomine*[1], *S. Mizuno*[1], *H. Furuumi*[1], *H. Shirozu*[1], *N. Hatano*[1], *T. Iwaki*[2], *Y. Jinno*[3], *H. Sasaki1.* [1]*Division of Disease Genes, Institute of Genetic Information;* [2]*Department of Neuropathology, Neurological Institute, Faculty of Medicine, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan.* [3]*Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan.*

A32. **A NEW RAT MUTATION WITH CEREBELLAR VERMIS DEFECT (cvd) MAPS TO CHROMOSOME 2.**

M. Kuwamura, *A. Takada, J. Yamate, T. Kotani, S. Sakuma, K. Kitada*, T. Serikawa*. Department of Veterinary Pathology, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan, *Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.*

A33. **COMPARISON OF THE GENE STRUCTURE OF THE MOUSE AND HUMAN NEURONAL β2-NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) GENES AND PHYSICAL MAPPING OF THE HUMAN GENE.**

Kira K. Lueders[1], *Ingo Marenholz*[2], *Dietmar Mischke*[2], *Dean Hamer*[1]. [1]*Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.* [2]*Institute for Immunogenetics, Charite, Humboldt University of Berlin, D-14050 Berlin, Germany.*

A34. **MAPPING OF THE TYPE 1 DIABETES LOCUS, *Idd3*, TO A 780 Kb INTERVAL ON PROXIMAL MOUSE CHROMOSOME 3.**

Paul A. Lyons, *Mary Beth Wilusz‡, Nicola Armitage, Patricia L. Podolin*, Christopher J. Lord, Paul Denny, Natasha J. Hill, John A. Todd, Linda S. Wicker*, Laurence B. Peterson‡. The Wellcome Trust Centre for Human Genetics, Nuffield Department of Surgery, University of Oxford, Headington, OX3 7BN, UK; the Departments of Autoimmune Diseases Research* and Cellular and Molecular Pharmacology‡, Merck Research Laboratories, Rahway, New Jersey 07065, USA.*

A35. **GENETIC AND PHYSICAL MAPPING OF *loop-tail*, A MOUSE MUTANT WITH A SEVERE NEURAL TUBE DEFECT, AND ANALYSIS OF CANDIDATE GENES.**

Jennifer N. Murdoch[1], *Jane Eddleston*[2], *Nathalie Leblond-Bourget*[3], *Philip Stanier*[2], *Andrew J. Copp*[1]. [1]*Neural Development, Institute of Child Health, University College London, 30 Guilford Street, London, WC1N 1EH, UK..* [2]*Action Research Laboratory for Fetal Development, Imperial College School of Medicine, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London W6 0XG, UK.* [3]*Present address: Laboratoire de Génétique et Microbiologie, Université Henri Poincaré, Boulevard des Aiguillettes, BP 239, 54506 Vandoeuvre-Lès-Nancy, France.*

A36. **A HIGH RESOLUTION GENETIC AND PHYSICAL MAP OF *mdfw*.**

K. Noben-Trauth, *F. Enahora, J. H. Kim. Section on Murine Genetics, National Institute on Deafness and Other Communication Disorders, NIH, 5 Research Court, Rockville, MD 20850..*

A37. GENETIC MAPPING AND MODIFIERS OF THE *bronx waltzer* MUTATION.

C.S. Nogueira, *M. Cheong, A. Kelly, T. Bussoli, K.P. Steel.*

A38. SYNTENIC ORGANISATION OF THE MOUSE DISTAL CHROMOSOME 7 IMPRINTING CLUSTER AND THE BECKWITH-WIEDEMANN SYNDROME REGION AT CHR. 11p15.5.

Martina Paulsen[1,2], *Karen R. Davies*[2], *Lucy M. Bowden*[2], *Angela J. Villar*[2], *Wolf Reik*[2], *Jörn Walter*[1]. [1]*Max-Planck-Institut für Molekulare Genetik, Ihnestr. 73, D-14195 Berlin, Germany.* [2]*Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge, CB2 4AT, UK.*

A39. A COMBINED GENETIC AND PHYSICAL MAP OF DISTAL MOUSE CHROMOSOME 12.

Roy Riblet, *America Mauhar, Brendan Brinkman, Christophe Chevillard, Christopher Herring. Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, California 93037. email: riblet@cerfnet.com*

A40. MOLECULAR CHARACTERIZATION OF 10 GENES MAPPED ON THE MOUSE t-COMPLEX.

Yuri Sano[1], *Hiroshi Nakashima*[1], *Hiroyuki Fujiwara*[1], *Kuniya Abe*[2], *Karen Artzt*[3], *Minoru S. H. Ko*[1]. [1]*Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48202, USA.* [2]*Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan.* [3]*Department of Zoology, University of Texas Austin, Texas 78712, USA.*

A41. A 600 kb BAC CONTIG COMPRISING THE LOCI wobbler, Mor2, Otx1, TYPE IIB IAP INTEGRASE AND FIVE NEW TESTIS EXPRESSED GENES ON MOUSE CHROMOSOME 11.

Dirk Korthaus, Cora Thiel, Volker Schmidt, Karin Resch, Melanie Ronsiek, Harald Jockusch, **Thomas Schmitt-John**. *Developmental Biology Unit, University of Bielefeld, D-33501 Bielefeld, Fed. Rep. Germany.*

A42. EXPLOITING UNAFFECTED F2 AND F3 LITTERMATES OF HOMOZYGOUS MUTANTS WITH INFORMATIVE RECOMBINATION BREAKPOINTS: A SIMPLE STRATEGY TO AVOID LARGE BACKCROSS/INTERCROSS PROGENY AS APPLIED TO THE MOUSE MUTATION wobbler.

Volker Schmidt, Martin Augustin[1], *Dirk Korthaus, Harald Jockusch,* **Thomas Schmitt-John**. *Developmental Biology Unit, University of Bielefeld, D-33501 Bielefeld, Fed. Rep. Germany.* [1]*present address: Fraunhofer-Institute, Department of Cell Biology, D-30625 Hanover, Fed. Rep. Germany.*

A43. POLYMORPHISMS AT THE CCT4 LOCUS ON MOUSE CHROMOSOME 11: APPLIED FOR A INTRA-STRAIN DIAGNOSIS OF THE wobbler (wr) GENE.

V. C. Schmidt, D. Korthaus, M. Augustin[1], *H. Jockusch,* **T. Schmitt-John**. *Developmental Biology, University of Bielefeld, D-33501 Bielefeld, Germany.* [1]*Present Address: Fraunhofer Institute of Toxicology and Aerosol Research Center for Medical Biotechnology and Department of Cell Biology, Nikolai-Fuchs-Str. 1, D-30625 Hannover, Germany.*

A44. LINKAGE ANALYSIS OF THE REPEATED EPILATION (*Er*) REGION ON MOUSE CHROMOSOME 4 REVEALS SEX AND STRAIN-SPECIFIC DIFFERENCES IN RECOMBINATION FREQUENCIES.

Rebecca A. Liddell[1], *J. Michael Wingrave*[1], *Jill K. Fisher*[1], *Renato V. Iozzo*[2], *Arthur M. Buchberg*[1], **Linda D. Siracusa**[1]. [1]*Department of Microbiology and Immunology, Kimmel Cancer Center;* [2]*Department of Pathology, Anatomy and Cell Biology, Jefferson Medical College, 233 South 10th Street, Philadelphia, PA 19107 USA.*

A45. MAPPING OF A LUNG TUMOR SUSCEPTIBILITY GENE WITHIN THE CLASS III REGION OF THE MOUSE MHC.

Margriet Snoek, *Huub van Vugt. Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam.*

A46. A PHYSICAL MAP OF THE MOUSE X CHROMOSOME.

P.J. Trickett[1], *P. Denny*[1], *T. Marsland*[1], *C. Heuston*[1], *A. Southwell*[1], *S. Greenaway*[1], *M.A. Strivens*[1], *M. Cadman*[1], *A.R. Haynes*[1], *D. Henriques*[1], *S. Jackson*[1], *Y.Y. Lau*[1], *P. Middlehurst*[1], *N.A. Quaderi*[1], *P. Weston*[2], *M. Ross*[3], *N. Carter*[3], *S.D.M. Brown*[1]. [1]*MRC UK Mouse Genome Centre and Mammalian Genetics Unit, Harwell, OX11 ORD, UK.* [2]*MRC Human Genome Mapping Project Resource Centre, Hinxton, Cambridge, UK.* [3]*The Sanger Centre, Hinxton, Cambridge, UK.*

A47. GENETIC ANALYSIS FOR SPONTANEOUS TONIC-CLONIC SEIZURES IN NER RAT STRAIN.

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A48. ANALYSIS OF SYNTENIC RELATIONSHIP BETWEEN MOUSE CHROMOSOME 9 AND HUMAN CHROMOSOME 6 BY RADIATION HYBRID MAPPING.

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A49. MOLECULAR AND GENETIC ANALYSIS OF LURCHER (*Lc*) AND PURKINJE CELL DEGENERATION (*pcd*) MUTATIONS.

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A8. A NEW MEMBER OF THE P53 FAMILY: MOLECULAR CLONING AND CHROMOSOMAL MAPPING OF THE GENE CODING FOR THE HUMAN TRANSCRIPTION FACTOR KET AND ITS MURINE HOMOLOG.

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KET is a member of the recently discovered family of proteins that is related to the transcription factor p53, which is known to play a major role in cell-cycle control and apoptosis. Loss of function mutations in the *p53* gene result in silenced tumor suppression and therefore, enhanced susceptibility to cancer development.

Mice deficient for the *p53* gene at one or both alleles develop cancers at an early age, but are overtly indistinguishable from wildtype during embryo- and organogenesis. However, in different p53 knockout lines a subset of *p53*-deficient embryos expressed a pathological phenotype characterized by abnormal head and brain morphology, the severity of which is modified by the genetic background on which the null-alleles were maintained. This indicates in different genetic backgrounds the existence of different compensation efficiencies towards the *p53* loss and the existence of gene products which accomplish function of p53 at least during embryogenesis.

Good candidates with such a compensatory mode of operation might be the so far discovered members of the p53 family, i.e. KET, originally cloned from rat, and the human p73 protein. These two genes encode putative transcription factors showing strong homology to p53.

Here we report the predicted amino acid sequence of the human KET protein in comparison to rat KET, human p53 and p73 proteins. The structure of KET as a transcription factor will be discussed. Furthermore, we present mapping data for human and murine KET/*Ket* genes, indicating in both species a possible role of KET in tumor suppression.

A9. A NOVEL MUTATION SHABBY (*Shby*) MAPS TO CHROMOSOME 17.

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Shabby (*Shby*) is a spontaneous, fully penetrant, dominant mutation which affects the skin, hair and growth of affected animals. *Shby/Shby, Shby/+* and *+/+* littermates can be distinguished at birth by the morphology of their vibrissae. Fur development is abnormal in both homozygotes and heterozygotes - the first growth of hair being thinner than normal. After the first moult the fur becomes very sparse; hair growth and loss continues in cycles, but becomes less pronounced with increasing age. The skin of affected animals is abnormal with the collagen being loose and fragmented, hair follicles are often plugged with keratin and have reduced levels of pigment; mast cells are also present in unusually high numbers in the dermis. There is no significant difference in weight at birth between affected and unaffected littermates, but at three weeks of age *Shby/+* are approximately 80% and *Shby/Shby* 65% the weight of normal littermates. These differences become less pronounced with increasing age. Heterozygotes show normal viability, but intercrosses between heterozygotes have yielded fewer than expected homozygotes at weaning. *Shby/+* and *Shby/Shby* males are fertile, but *Shby/+* females have a slightly reduced litter size. Cytogenetic studies revealed no obvious anomaly.

Shby has been assigned to chromosome 17 with a two point linkage with brachyury (*T*). Outcrossing *T Shby /++* males and females gave the following progeny: 111 *T Shby*, 121 +, 12 *T*, 15 *Shby* , outcrossing *T +/+ Shby* males and females yielded: 350 *T*, 334 *Shby*, 37 *T Shby*, 37 +; the combined data placing *Shby* 10.8 ± 1.0 cM from *T*. To position *Shby* more precisely a four point linkage test was performed with *T*, quaking (*qk*) and tufted (*tf*). *tf* mice have a normal appearance prior to weaning, but fur loss is obvious by four weeks of age; *tf* could not be scored in *Shby* mice. Crosses of *T qk tf +/+++ Shby* males to *qk tf* homozygous females gave 43 *T qk tf*, 60 *tf? Shby*, 1 *T tf? Shby*, 4 *tf*, 4 *T qk tf? Shby*, 2 +; crosses of *T ++ Shby/+ qk tf +* males to *qk tf* homozygous females gave 22 *qk tf*, 25 *T tf? Shby*, 1 *Shby*, 2 *qk tf? Shby*, 1 *T*. To calculate the genetic distance between tf and Shby, only the $+^{Shby}$ were used. This gave a gene order of *T, qk, tf, Shby* since any other order would require double crossovers to explain the offspring; *Shby* lying 4.2 ± 2.36 cM distal to *tf*. No other mutations affecting fur loss have previously been mapped to this region.

A10. THE JACKSON LABORATORY MAPPING PANELS: BACKCROSS AND RADIATION HYBRID DATABASES.

Mary Barter, *Lucy Rowe, Michael Rourk, Juergen Naggert, Janan Eppig.*

The Jackson Laboratory Backcross DNA Panel Mapping Resource maintains and distributes small aliquots of DNA from two panels of 94 backcross animals: one from the cross (C57BL/6J x *M. spretus*) x C57BL/6J, called BSB, and the other from the reciprocal cross (C57BL/6JEi x SPRET/Ei) x SPRET/Ei, called BSS. These DNAs were prepared from whole animals, yielding a large stock of DNA, and therefore we are able to map a very large number of loci over time. We are providing these DNAs to other investigators interested in mapping loci, either as 125 ng/µl aliquots of DNA to be diluted for use as PCR templates, as 0.5 µg/µl aliquots of DNA for Southern blot analysis, or as Southern blot filter sets (please inquire).

We also are typing large numbers of new loci on these backcross panels in order to increase their usefulness for mapping genes. The Panel maps are anchored to maps from other crosses by various known genes, retroviral loci, and with the D#Mit# SSLP loci available from Research Genetics. We have been adding D#Mit# loci with the goal of at least one MIT anchor locus every 5 cM throughout the maps. The number of loci mapped on the Panels is rapidly increasing, with more than 870 loci on the public BSB map and 3720 on the BSS map in June 1998. In the past year, 1194 new loci have been mapped with these crosses. All new loci typed are localized readily within these maps. New data are being contributed by an increasing number of labs. The public data are available from from our World Wide Web site (http://www.jax.org/resources/documents/cmdata), the Mouse Genome Database (MGD), Portable Dictionary of the Mouse Genome, and to all users of the Mapping Resource. A complete report on the construction and preliminary characterization of the Panels was published in Rowe et al. 1994 *Mammalian Genome* 5: 253-274.

As a complement to this interspecific backcross mapping database, we recently have established a comprehensive database for the mouse **T31 Radiation Hybrid mapping data.** This new database operates in a similar manner to the backcross database by offering detailed analysis of newly submitted RH mapping data, with technical support, and WWW posting of data and maps. In June 1998 there were over 500 loci placed in the public RH database, from 34 laboratories. Most chromosomes are not yet densely enough mapped to achieve significant linkage along their entire length. Maps are presented in this case in the locus order from the available genetic mapping data. (See associated abstract on RH Mapping Database by Rowe et al.).

This work is supported by NIH grant HG00941.

A11. SEQUENCING THE MOUSE Y CHROMOSOME SHORT ARM.

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In addition to its essential function in sex determination, it has been shown that the small (~5Mb) short arm of the mouse Y (Yp) encodes genes which play an indispensable role in spermatogenesis. A complete sequence analysis of mouse Yp would be highly desirable yielding the genomic structure of previously identified genes, identification of novel genes which may play a role male development and allowing sequence comparison with homologous regions of the human Y chromosome.

In order to achieve this our laboratory is converting our previous low resolution structural maps into a sequence ready BAC contig of this region of the Y which will be sequenced in collaboration with the BCM Genome Center. To date, we have constructed a bacterial artificial chromosome (BAC) contig in excess of 1.2Mb of genomic DNA encompassing the Sxrb deletion region of mouse Yp. We have started with this area as it has been shown to be particularly gene-dense. The map contains 30 overlapping BACs in two contigs with two gaps and has generated 25 new Y specific or XY common sequence tagged sites (STS).

The location of all BACs used for sequencing has been verified through the use of FISH analysis, PCR analysis using Y specific STSs and high resolution fingerprinting. All previously identified genes have been positioned on this map, providing further basis for the structural comparison of the mouse Y chromosome with that of other species, including man. As sequence data from this project becomes available from the Baylor College of Medicine Human Genome Sequencing Center, it will be available through mouse Y chromosome committee report WWW site.

A12. SEX-LINKED FIDGET, A MUTATION WHICH AFFECTS COCHLEAR DEVELOPMENT, IS ASSOCIATED WITH RECOMBINATION SUPPRESSION IN THE *DXMit109 -Pou3f4* REGION.

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Sex linked fidget (*slf*) arose in a radiation mutagenesis experiment which made use of the In(X)1H inversion to test for induced sex linked lethals (Lyon et al. 1982, *Mutation Research* 92: 217-228). The phenotype is inherited in a X-linked recessive manner with *slf*/Y and *slf/slf* animals exhibiting a mild vertical head-shaking which is most easily scored at weaning age. We have examined the inner ear structures from affected animals and observed gross abnormalities which included a lack of correct spiralling of the cochlea. Early mapping experiments suggested that *slf* was closely linked to tabby (Ta) which would place it in a region of the mouse X chromosome homologous to the Xq13 of the human X chromosome. To position *slf* more accurately we established a (*slf/slf* x *Mus spretus*) x 3H1 interspecific backcross but were unable to accurately score the phenotype in the backcross progeny. However, typing 110 backcross progeny with a range of X-linked markers revealed a region of recombination suppression which stretched from *DXMit109* to *Pou3f4*. Chromosomal rearrangements upstream of POU3F4 on the human X chromosome, as well as missense mutations, are known to be associated with the most common form of X-linked deafness (DFN3) in man (deKok *et al.* 1996, *Human Molecular Genetics* 5: 1229-1235; deKok *et al.* 1997, *Human Mutation* 10: 207-211). We suggest that the *slf* phenotype is caused by a chromosomal inversion with one breakpoint close to *Pou3f4* and are currently investigating this by *in situ* hybridisation.

A13. GENETIC AND PHYSICAL CHARACTERIZATION OF THE REGION AROUND *waltzer* ON MOUSE CHROMOSOME 10.

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Albany waltzer (v^{Alb}) is an allele of waltzer, a deafness gene on mouse Chromosome 10. We have previously described high resolution mapping of v^{Alb} to an approximately 1 cM region flanked by *D10Mit60* and a new marker (5'40) generated from a bacterial artificial chromosome (BAC) in the waltzer critical region. Recent efforts have focused on physically mapping the *v* gene by isolating a series of overlapping yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) clones from the region around waltzer. Additionally, a newly available mouse radiation hybrid (RH) panel is being used as a complementary tool for refining the genetic map of the *v* critical region. Several genes, including sphingosine-1-phosphate lyase (*spl*) and slowpoke (*mSlo*) are being mapped and assessed as possible candidates for the *v* gene. Finally, BACs in and around *waltzer* are being subcloned to obtain nucleotide sequences for possible identification of new genes and markers in the *v* critical region.

A14. AMES waltzer (av): PHYSIOLOGICAL AND HISTOLOGICAL CHARACTERIZATION AND GENETIC MAPPING TO MOUSE CHROMOSOME 10 AT THE BORDER OF 10q21 AND 22q11 SYNTENY.

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The Ames waltzer (*av*) mouse mutant is an autosomal recessive deafness mutation on mouse Chromosome 10. In order to understand the phenotype, we have performed functional and histological characterization of all 3 alleles of *av*. av^{2J} is more severe than av^{J}: mice are more hyperactive, can't swim, head toss and circle, and young av^{2J} mice walk unsteadily, indicating vestibular involvement, whereas avJmice occasionally circle, but do not usually toss their heads and some can swim. No allele of *av* ever shows any evidence of hearing at any age or frequency. The endocochlear potential (EP) is normal, indicating *av* to be a neuroepithelial mutation. Histologically, degeneration of the neuroepithelium, lack of spaces of Nuel, and deficient or absent opening of the tunnel of Corti are observed. The time course of hair cell degeneration shortly follows the normal development from base to apex.

We have performed an intersubspecific backcross with *Mus musculus castaneus*, and mapped microsatellite markers in an *av* cross. Toothpick PCR on previously frozen tissue samples from offspring was used as an efficient strategy to quickly screen a large number of animals. In 1258 progeny tested we found 3 recombinants for each of the flanking markers *D10Mit199* and *D10Mit64*. In addition, nine different genes (*Ank3, Bcr, Gnaz, Tfam, Mif, Mmp11, Dcoh, Pyp and Gstt2*) were mapped and eliminated genetically as candidate genes for *av*. Our mapping data indicate that av maps near an evolutionary break, between synteny to 22q11 and 10q21. The orientation of these two human chromosomal regions relative to mouse Chromosome 10 was determined. *av* has been discussed as a potential model for human Usher syndrome type ID (USH1D) and DFNB12. However, based on our data these are unlikely to be human homologues of *av*. In contrast, *USH1F* maps to the region homologous to av. Physical mapping of the region in BACs and YACs, as well as mapping of candidate genes and ESTs will help us to isolate the *av* gene. Supported by the NIDCD (DD, YR and MB).

A15. COMPARATIVE AND PHYSICAL MAPPING OF CONSERVED LINKAGE REGIONS WITH HSA21 AND IMPLICATIONS FOR DS MODELS.

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Human Chromosome 21 (HSA21) includes regions of conserved linkage with mouse Chromosomes (MMU) 10, 16, and 17. High resolution physical maps have been constructed spanning 8.7 MB of MMU10 and 16 to aid in development of mouse models for Down Syndrome (DS). YAC and BAC/PAC contigs have been constructed across the distal 4.5 Mb of MMU16 and physical distances have been determined by analysis of more than 300 YAC fragmentation derivatives. The map has an average marker density of 1 marker/ 66kb. Twenty-two transcripts have been mapped in this interval in both species and there are no discordancies with gene order on HSA21. Comparison to HSA21 physical maps and genomic sequence shows that the proximal 3 Mb covered by the contig is conserved in size between the two species, while the distal 1.5 Mb of MMU16 is compressed compared to HSA21. The MMU10 contig spans the 2.0 Mb of HSA21 conserved synteny from *Cstb* to *Prmt2*, and crosses into a region of MMU10/HSA22 homology, thus cloning the junction and adding another 1.2 Mb of coverage on MMU10. The MMU10 contig contains 22 expressed sequences, 12 SSRs and 16 anonymous DNA markers (a density of 1 marker/73 kb). Within this region of MMU10/HSA21 homology the mouse physical map is considerably compressed compared to human physical maps.

Sequence ready template is being assembled for both regions by hybridizing gel-purified, minimal tiling path YACs to high density filter arrays of PACs (BAC/PAC Resource, RPMI) and determination of contig order by fingerprinting, STS content mapping and end clone recovery. More than 4 MB of sequence ready template is in place including a contiguous span of more than 1.5 MB on MMU16 and more than 2.0 MB of 6-deep template on MMU10. Sequencing is ongoing for PACs that cross the evolutionary breakpoint between HSA21 homology and HSA22 homology (B. Roe, U. Oklahoma - see Lund et al.) and for several segments of MMU16 for which HSA21 sequence is available.

The results of this work are immediately applicable to the refinement of mouse models of DS. Microinjection of overlapping PACs is being used to create mice that are transgenic for defined regions of the conserved linkage. Cre-mediated chromosomal engineering has been initiated to create mice that are duplicated or deleted for the entire region of conserved linkage between HSA21 and MMU10.

A16. PHYSICAL MAPPING WITHIN THE UROGENITAL SYNDROME REGION ON PROXIMAL MMU2.

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The urogenital syndrome (*us*) mutation is a naturally occurring mouse mutation which affects the axial skeleton and the urogenital system. This mutation arose on a linkage cross, and was maintained by a series of outcrosses to increase fertility and viability. Linkage analysis indicates that the mutated gene underlying this defect is located on proximal chromosome 2, between the genes *Pax8* and *Spna2* (Lane and Birkenmeier, 1993).

Our interests are to further characterize the phenotype produced by this mutation and to identify the underlying molecular defect. Characterization of the phenotype will begin when these mice have been recovered from cryopreservation. However, since this mutation has been localized between two genes, analysis of this region has been initiated.

We have begun characterization of this region by integration of existing genetic maps. Ten SSLP markers generated at the Whitehead Genome center have been mapped on one or both backcross panels, BSS or BSB, generated at the Jackson Laboratory. In addition, five markers which had previously been mapped only on the BSS panel were placed on the BSB panel. We have also begun physical mapping using Radiation Hybrids (RH mapping), YAC STS content, and analysis of large deficiency complexes generated using the *Cre/LoxP* recombinase system. To date, 37 markers have been localized to this 4 cM region, thus giving a density of approximately 1 STS/0.1 cM. Presently 22 of these markers have identified 24 YAC clones, and 13 STSs have been placed on the RH map. Ten deficiencies have been produced which are anchored around the Notch locus. Two of these show LOH only for Notch, two show LOH for three loci, two show LOH for five loci, and two show LOH for six loci. Additional mapping information for this region will be presented.

Characterization of this region will be useful for the identification of other mutations which also map to this region, including sarcosinemia, stubby, and ebouriffe. In addition, this region shows a large degree of conserved synteny with human 9q34, a gene rich region which contains many disease causing mutations in humans.

A17. AN 85KB TANDEM TRIPLICATION IN THE SLOW WALLERIAN DEGENERATION (*Wld*^{2}) MOUSE.

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Wallerian degeneration is the degeneration of the distal stump of an injured axon. This normally occurs over a time course of around 24 hours but it is delayed in the C57BL/*Wlds* mouse for up to three weeks. The neuroprotective gene, *Wld*, has previously been mapped to distal chromosome 4. Here we report the fine genetic and physical mapping of the *Wld* locus and the identification of an 85kb tandem triplication mapping within the candidate region. The mutation is unique to C57BL/*Wlds* amongst 36 strains tested and is therefore a strong candidate for the mutation which leads to delayed Wallerian degeneration. It is likely to influence the *Wld* gene through increased gene dosage, disruption of the gene at the repeat unit boundary/ies or a position effect.

There are very few reports of tandem triplications in a vertebrate and no evidence for a mutation mechanism so this unusual mutation was characterized in more detail. Sequence analysis of the boundaries of the repeat unit revealed a novel minisatellite array at the distal boundary and a matching 8bp sequence at the proximal boundary. This suggests that recombination between short homologous sequences ("illegitimate" or "non-homologous" recombination) was involved in the rearrangement. Furthermore, a duplication allele was identified in two Wlds mice, indicating some instability in the repeat copy number and suggesting that the triplication arose from a duplication by unequal crossing over.

A18. CHROMOSOMES IN THE BARE PATCHES AND STRIATED CRITICAL REGION.

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Bare-patches (*Bpa*) and Striated (*Str*) are X-linked dominant disorders that are lethal in hemizygous male mice and may have human homologs in chondrodysplasia punctata type 2 (CDPX2) and incontinentia pigmenti (IP2), respectively. The murine loci have been mapped to a 600kb region that is homologous to human Xq28. Exon trapping and cDNA selection using cosmids which map to the *Bpa/Str* critical interval have identified a number of novel candidate genes. To construct a complete transcription map, we have undertaken genomic sequencing of the entire region using a combination of cosmid, BAC and PAC templates. Over 500 kb of mouse sequence in contigs >1kb has been accumulated, including finished sequence for a cosmid (EMBL accession no. AL021127) and four BACs (ftp://www.hgmp.mrc.ac.uk/pub/mouseseq). We have also sequenced more than 630 kb in the corresponding region of human Xq28 (http://genome.imb-jena.de).

Our approach for identifying novel genes within this region is based on a number of techniques, including exon and gene-structure prediction, sequence database homology searches, pairwise alignments with human genomic sequence and isolation and analysis of cDNAs. We will present our results in identifying novel genes and also in testing the prediction accuracy of the exon and gene-structure prediction packages on mouse and human genomic DNA in the region.

This work is part of a larger project to test the value of comparative sequence analysis in the identification of genes and other genomic elements, such as enhancers (http://www.mgc.har.mrc.ac.uk/comp_seq/). We believe that this will be more productive and more sensitive than analysis of sequence from only one species. Together, we plan to sequence about 6 Mb of the mouse X chromosome, split into several regions between the *Ids* and *Dmd* loci (e.g. see abstract by Platzer et al). One of our next targets is between the *Cf8* and *Dmd* loci. It is relatively gene poor and crosses an evolutionary breakpoint. Significant portions of the corresponding human chromosomal regions have been or will soon be sequenced allowing us to perform a detailed comparative analysis across an evolutionary breakpoint region of a mammalian chromosome.

A19. SCURFY: A SEVERE X-LINKED IMMUNE DISEASE IN THE MOUSE.

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Scurfy is a fatal immune disease characterized by runting, swollen abdomen, scaliness of skin, ears and tail, and early death; typically by 3-4 weeks of age. Pathologically the mice display splenomegaly, lymphadenopathy, hypergammaglobulinemia, hypogonadism, a shrunken thymus and dermal thickening associated with massive mononuclear cell infiltration. A number of studies have highlighted the importance of T cells, specifically CD4+ T cells, in driving the disease.

Transplantation experiments have pointed to a central role for the thymus and have led to the hypothesis that scurfy is the result of a failure of T cell education in the thymus.

Our work has focused on trying to understand the biological nature of the disease coupled with attempts to clone the gene responsible. For the latter we have undertaken a positional cloning strategy, using BACs to clone the region genetically linked to the trait. From these BACs a large number of novel genes have been cloned. Currently we are employing functional complementation to narrow down the region containing the gene. This involves generating mice transgenic for each BAC and crossing these onto the scurfy background, thereby assaying the ability of individual BACs to complement the genetic defect. We will present our current progress towards isolating the gene responsible for scurfy.

A20. PHYSICAL MAPPING OF THE REGION ON MOUSE CHROMOSOME 7 HOMOLOGOUS TO HUMAN CHROMOSOME 15q11-q13.

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In concert with the directed mutagenesis efforts at Oak Ridge National Laboratory, in which single-gene mutations are being induced and mapped within regions covered by long, radiation-induced deletions at the mouse Chr-7 pink-eyed dilution (*p*) locus, we are building sequence-ready physical maps. With three dozen smaller, multilocus *p*-region deletions available for the simultaneous mapping of mutant phenotypes and large DNA clones into defined deletion intervals, we can assign functional information to genes identified by DNA sequencing.

One of the Oak Ridge radiation-induced p deletions undergoing such mutagenesis, *Df(Ery1 p Gabrg3 Gabra5 Gabrb3 Ube3a Ipw)*^{30Pub}, includes *Ube3a*, the putative Angelman Syndrome (AS) gene and *Ipw* (an imprinted transcript from the Prader-Willi Syndrome (PWS) critical region, as well as other genes that may play roles in neurological and physical wellness. The linkage order of known genes in this segment, extending from *Ery1* through *Znf127*, appears to be maintained in the homologous segment of human chromosome 15q11-q13, which includes imprinted genes contributing to PWS and AS.

We have built a 1.4Mb YAC contig distal to *p*, between *Gabrb3* and *Ipw*, and are constructing a sequence-ready BAC contig. Microsatellites were first fine-mapped within the *p* deletion complex, and then used to screen YAC and BAC libraries (Research Genetics Inc. and Genome Systems Inc.); additional probes have been generated to close gaps and increase coverage. Sequencing of mouse DNA clones in this region, in conjunction with the sequencing of the homologous 15q11-q13 region, will identify coding sequences that may play roles in AS, PWS, and other mutant phenotypes uncovered by the ongoing mutagenesis/phenotype-screening experiments.

A21. NEW REARRANGEMENT BETWEEN MOUSE AND HUMAN X CHROMOSOMES: TBL1 MAPS AT THE INTERFACE BETWEEN TWO LARGE REGIONS OF CONSERVATION.

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The genetic content of the X chromosome is highly conserved in eutherian mammals but evolutionary rearrangements have resulted in blocks of conserved regions best defined between human and mouse. Recently we reported the mapping of the murine homologs of APXL and OA1, two genes located at band Xp22.3 in human, to band F2-F3 of the mouse X chromosome. We now have mapped the mouse homolog of TBL1, a gene also located at band Xp22.3 in human in very close proximity to APXL and OA1. Interestingly, the mouse *Tbl1* gene maps to band B-C in both a laboratory strain of mouse and in *Mus spretus*, in a recombination interval between the mouse homologs of CF8, located at band Xp28 in human, and DMD, located at band Xp21.3-21.2.

This result which defines yet another region of conservation between human and mouse X chromosomes, emphasizes the high rate of evolutionary rearrangements involving markers at the distal end if the human X chromosome. The mouse *Tbl1* gene, like *Oa1* and *Apxl*, is located at the interface between two large blocks of conservation, suggesting that insertions of shorter genomic regions may characterize the boundaries between larger regions of conservation.

A22. THE *fused phalanges* ALLELE OF *shaker-with-syndactylism* MAPS TO MIDDLE-DISTAL MOUSE CHROMOSOME 18, AND SPANS A REGION CONTAINING GENES SYNTENIC WITH HUMAN CHROMOSOME 5q.

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fused phalanges (*sy*^{fp}) is an allele of *shaker-with-syndactylism* (*sy*) (Lane *et al.*, 1973, *Mouse News Lett.* 49: 32). *sy*^{fp}/*sy*^{fp} and *sy/sy* mice have syndactylous fore and hind feet (Hummel *et al.*, 1971 *Mouse News Lett.* 45: 28). In addition *sy/sy* mice have abnormally developed sacral vertebrae, scapula, and long bones (Grüneberg, 1962, *Genet. Res.* 3:157-166). Morphogenetic malformation of the inner ear and circling behaviour, indicative of vestibular dysfunction, have been noted in *sy/sy* mice (Deol, 1963 *J. Embryol. Exp. Morphol.* 11: 493-512). The compound mouse *sy/sy*^{fp} resembles *sy*^{fp}/*sy*^{fp}. This suggests that *shaker-with-syndactylism* may be a contiguous gene deletion syndrome.

sy^{fp}/*sy*^{fp} offspring have a higher level of viability and fertility than *sy/sy* (Lane *et al.*, 1981, *J. Hered.* 72: 409-12); thus the *sy*^{fp} allele facilitates quicker mapping of the *shaker-with-syndactylism* locus. In the present study, we have genetically mapped *sy*^{fp} using 58 intraspecific backcross progeny ([*sy*^{fp}/*sy*^{fp} x C57Bl/6J]F1 x *sy*^{fp}/*sy*^{fp}) to middle-distal mouse chromosome 18, establishing the following locus order: *Centromere - D18Mit17 - 5.2cM ± 2.9 - [D18Mit74, D18Mit124, sy*^{fp}*] - 6.9cM ± 3.3 - D18Mit40 - 3.5cM ± 2.4 - D18Mit183 - 10.3cM ± 4.0 - [D18Mit154, D18Mit79] - 3.5cM ± 2.4 - D18Mit48.*

Interpolation of these data with other genetic maps indicates that the location of *sy*^{fp} spans a region which contains genes syntenic with human chromosome 5q (Dietrich et al., 1996, *Nature* 380: 149-151, Pataer *et al.*, 1996, Cancer Res. 56: 3716-3720, EUCIB Mouse Chromosome 18 Linkage Map located at *http://hgmp.mrc.ac.uk/MBx/MBxhomepage.html/*, and SELDIN Mouse Chromosome 18 Linkage Map, located at *http://informatics.jax.org/*).

Establishing the conservation of human synteny, and our continuing genotyping of further backcross progeny, may facilitate a candidate gene approach towards gene isolation, or permit the development of a physical map. The localisation of the genes involved in *shaker-with-syndactylism* will advance our understanding of both inner ear and skeletal development.

This study has been supported by the Medical Research Council.

A23. FINER MAPPING OF THE MOUSE MUTATION CALLED DOE WITH NEUROLOGICAL, EYE, AND COAT COLOR DEFECTS.

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Chromosomal aberrations, such as deletions, inversions and translocations, have been induced in post-meiotic male germ cells by the chemotherapeutic agent chlorambucil. One resulting mouse mutant, called *Doe*, was discovered by its dominant phenotype of open eyelids at birth. Previous studies mapped this mutation to both chromosomes 2 and 13. Cytogenetic analysis confirms that this mutation maps to the site of a reciprocal translocation between chromosomes 2 and 13. In addition to its open eyelids, the *Doe*/+ mouse exhibits abnormal neurological behavior consisting of star-gazing, hyperactivity and occasional circling. When placed on a pigmented background, the heterozygous mice are dilute in color indicating a pigmentation defect as well. The translocation breakpoint, presumably causing this mutation, resides on the very proximal part of chromosome 2 and on chromosome 13 near the SSLP marker *D13Mit64*. With the availability of additional SSLP markers, we are now more precisely mapping the position of this mutation/translocation.

A24. MAPPING OF A MOUSE MOTOR NEURON DISEASE GENE, LOA, TO THE DISTAL REGION OF MMU12.

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Motor neuron disease (MND) is a progressive genetic disorder that has an incidence of 2 in 100,000 and a prevalence of 5 in 100,000. Mouse models of human genetic disorders are valuable tools for identifying the genes that are involved in disease and elucidating the function of the genes products. We have been using such a mouse model for the human MND. This mutant mouse is called Loa, for legs at odd angle, and shows aspects of phenotype observed in human motor neuron disease.

We set up a large intraspecific backcross between the Loa mutant mouse and the inbred strain C57BL/6. Only affected N1 mice were then backcrossed to C57BL/6 to generate more than 1,000 N2 affected animals. Using these N2 mice, we have mapped the Loa mutation to the distal region of MMU12. The Loa critical region is flanked by D12Mit17 and D12Mit181, spanning approximately 1.6 cM of this region.

At present we are constructing a YAC contig of the region to further narrow down the critical region and follow a typical positional cloning strategy to isolate the Loa gene.

A25. MOUSE CHROMOSOME 4: A "HOT CHROMOSOME" FOR THE LOCATION OF TUMOR SUPPRESSOR GENES.

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In a previous paper we described a LOH study on the mouse chromosome 4 in γ-radiation-induced T-cell lymphomas of (C57BL/6J x RF/J) hybrid F1 mice, using polymorphic microsatellites (Santos et al., 1996. Oncogene,12, 669-676). That study suggested the existence of a critical region of LOH of about 0.6 cM between the markers *D4Wsm1* (*Ifa*) and *D4Mit9*, designated as TLSR1 (Thymic Lymphoma Suppressor Region 1). Intriguing evidence for a more distal region centered at the *D4Mit54* (TLSR2) was also provided. $p16^{INK4a}$ and $p15^{INK4b}$ analysis in the same tumors demostrated frequent transcriptional inactivation and DNA hypermethylation of these two genes, as well as a strong correlation between samples with LOH in TLSR1 and those showing LOH at $p15^{INK4b}$ (Malumbres et al., 1997. Oncogene, 14, 1361-1370). A more detailed allelotype analysis in the mentioned tumors, as well as in T-cell lymphomas of (C57BL/6J x BALB/cJ) F1 hybrids, with new microsatellites distributed along the chromosome 4, allowed us: (1) to confirm the existence of TLSR2 and to define it more precisely as centered at *D4Mit205b*; (2) to define a new candidate region on the distal part of chromosome 4, located between the *Mom-1* locus and *D4Mit68* (TLSR3); (3) to identify a novel region proximal to $p16^{INK4a}$ and $p15^{INK4b}$ and centered at *D4Mit116* (TLSR4); and (4) to demonstrate the presence of a new region on the proximal part of chromosome 4, located between *D4Mit21* and *D4Mit151* (TLSR5). These regions are syntenic with the human chromosomes 1, 6, 8 and 9 in where have been located tumor suppressor gene regions in a wide variety of tumors. The accumulation of candidate sites for suppressor genes in mouse chromosome 4 is an indication of the main role that plays this chromosome in the development of murine tumors.

A26. RADIATION HYBRID MAPPING OF MOUSE EXPRESSED SEQUENCE TAGS (ESTs).

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The laboratory mouse is an extremely useful model for the study of human genetic disease. However, the mouse mutant resource encompasses mutations at only 1-2% of the total number of mammalian genes - there is a phenotype gap (Brown and Peters, 1997). This deficiency is being addressed here at Harwell and by others in the application of systematic screening of ENU induced mutations (e.g. see abstract by Nolan et al).

There is a further deficiency in mouse genomics: the lack of a high-density transcript map. Such a map would be invaluable when positionally cloning mutant genes. Several groups, including our own, are working on producing transcript maps using DNA from a panel of mouse:hamster radiation hybrid (RH) cell lines (Research Genetics). Our approach is based on the design of PCR assays from 3, expressed sequence tags (3, ESTs) which work on genomic DNA templates. We are screening the RH panel using a Biomek 2000 pipetting robot to set up PCRs and analysing the products on standard agarose gels.

We are targeting those mouse ESTs that appear to lack human homologs. This approach is based on the likelihood that these genes will have human equivalents, but due to bias in the choice of cDNA libraries, they have not yet been sequenced. In this way, we will complement earlier and ongoing efforts in mapping human ESTs.

Along with other European groups, we are initially focusing on the construction of a robust radiation hybrid framework map that will be used for the mapping of novel ESTs. Database analysis of ESTs from a 3.5dpc blastocyst library indicates that around 30% do not have anidentifiable human equivalent (see abstract by Ko et al). We will present our progress in framework construction and EST mapping.

A27. HYBRIDIZATION-BASED APPROACHES FOR INTEGRATED MOUSE GENOME MAPPING.

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We present two different strategies for mapping large insert genomic clones in the mouse genome (1) IRS-PCR and (2) a modification of AFLP. IRS-PCR is based on the amplification of unique sequences that are flanked by repeat elements and can be performed on any reagent that contains genomic mouse DNA, i.e. DNA isolated from mouse tissue, somatic cell hybrids including radiation hybrids and large insert YAC and BAC/PAC clones. Individual fragments from BAC/PAC or YAC clones can be hybridised against Southern blots or robotically-prepared filters that contain complex PCR samples produced from individual backcross mice, cell hybrids or YAC-pools. Our progress in global mouse mapping integrating genetic and physical mapping data and providing links to the mouse proteome characterization will be presented.

In our modification to AFLP, amplicons are generated by ligation of double stranded adaptor molecules to genomic DNA cleaved with a restriction enzyme. Using primers that extend beyond the restriction site, reduced-complexity subsets of fragments are generated by PCR. Differences in the composition of complex probes generated from DNA of different strains are revealed through hybridization against high-density filter grids of large-insert genomic clones. Genetic mapping of genomic clones is achieved by hybridizing complex probes derived from backcross animals against the polymorphic clones. We would expect the method to be of particular use to generate markers for species that have not yet been extensively studied as a substantial number of easy-to-use markers can be recruited in a relatively short period of time.

A28. MAPPING THE Hsc70, Hsc74, AND Hsp70RY MEMBERS OF THE MOUSE HEAT SHOCK PROTEIN FAMILY AND AN APPARENT LACK OF MOUSE HOMOLOGUES TO THE HUMAN Hsp70 HSPA6/7 GENES.

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Mammalian cells respond to elevated temperatures by inducing or increasing the synthesis of a group of proteins called heat shock proteins (HSPs). These proteins are further subdivided into families according to their approximate molecular weights into HSP110, HSP90, HSP70 and HSP28. Within each classification multiple genes have been identified by DNA sequence analysis only some of which are heat inducible. Previous mapping studies have identified a three gene cluster of HSP70 genes on mouse chromosome 17. To search for similar clusters we have mapped the locations of 3 addition HSP genes by Southern blot analysis of genomic DNA from somatic cell hybrids.

Previous mapping of the human *Hsp70RY* and *Hsc74* genes (Fatahallah et al. 1993, Kaul et al. 1995) by in situ hybridization with cDNA probes located homologous regions for both genes at 5q31.1. Isolation of the corresponding mouse genes followed by Southern blot analysis located the mouse *Hsp70RY* gene to Chr11 near the *Gm2a* gene while the *Hsc74* gene was located on Chr 18 near *CamK4*. In addition, a psuedogene was located on the X chromosome, though in some mouse strains a copy on Chr 8 was also found. Examination of the mouse-human chromosome homology maps indicates 5q31.1 in humans corresponds with the junction between mouse Chrs11 and 18. Similar analysis of the mouse *Hsc70* gene located it to Chr 9 near the *Olfr7* locus. This region is syntenic with 11q23 in humans where a human *Hsc70* gene was mapped by in situ hybridization. To date most mouse *Hsp70* genes have been found to have corresponding human homologs. However, in humans two heat inducible *Hsp70* genes (HSPA6 and HSPA7) have been isolated for which no corresponding mouse genes are known. The human HSPA6 and HSPA7 genes were isolated and both mapped by in situ hybridization to 1q21.3. Detailed restriction mapping and DNA sequence analysis indicated the HSPA7 gene was non-functional. DNA probes from the HSPA6 gene, which should have cross-hybridized to any related mouse genes, did not detect any such gene. This leads us to conclude that while all mouse *Hsp70* genes known to have human homologs map to regions syntenic with their human counterparts there has been an expansion of the human *Hsp70* gene family which has not occurred in the mouse.

A29. CHARACTERIZATION AND GENETIC LOCALIZATION OF *strigosus (stg)* and *longjohn (lgj)*, NEW MUTATIONS AFFECTING GROWTH AND BODY SHAPE.

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Strigosus (stg) is a new recessive mutation of the mouse discovered in the progeny of an ENU (ethylnitroso urea) treated male. *Longjohn (lgj)* is a new recessive mutation that arose spontaneously at The Jackson Laboratory. Homozygous *stg* and *lgj* mice are first recognized at 6-8 days of age because of an increase in body length. Adults have tail kinks and exhibit kyphosis and arachnodactyly. Both *stg/stg* females and males are fertile whereas only *lgj/lgj* females are fertile. Skeletal stainings performed at different stages of development with alizarin red and alcian blue indicate that the ossification process in *stg/stg* mice is slightly delayed. Morphometric analysis is in process.

The *longjohn* mutation had been mapped to the proximal region of Chr 15. Given the similiarity of phenotype causes by *stg* and *lgj*, *stg* was tested for linkage with proximal Chr 15 markers. The results were positive. Allelism tests confirmed that *stg* and *lgj* are alleles. Further mapping experiments placed *stg/lgj* within in a 1 cM interval on Chr 15 between the *D15Mit10* and *D15Mit53*.

Positional cloning experiments are now underway. 11 YACs and 6 BACs have been isolated using flanking markers and a contig encompassing the 1 cM interval is being established. ESTs that map to this region of Chr 15 are being explored as possible candidate genes but as yet none have cosegregates with the mutant gene in the backcross. To date we have not been able to utilize human ESTs as possible candidate genes because linkage homology between the Chr 15 segment containing *stg/lgj* and a human chromosomal segment is not established.

A30. GENE CONTENT IN A FULLY-SEQUENCED 175 kb BAC CLONE FROM THE t-COMPLEX ON CHROMOSOME 17: 11 GENES INCLUDING *Nbps*, *Als* AND *D17Wsu15e*.

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In the process of characterizing a cDNA library prepared from the ectoplacental cone of 7.5 d.p.c mouse embryos, a number of genes encountered as expressed at a high level were mapped to the t-complex region of mouse chromosome 17. To place and help characterize many of the transcripts, including candidates associated with t-complex lethal mutants, we have begun an interactive study of their genomic and cDNA forms. We are constructing an STS/BAC map to integrate existing map data and provide substrates for the sequencing of 10 Mb of the region. From the initially targeted 1 Mb of overlapping BAC clones, the sequencing of one 175 kb clone has been completed with greater than 99.9% accuracy. Computer-assisted analysis of the sequence using GRAIL suggests 111 candidate exons, with an additional 300 potential candidate exons suggested by a less conservative program (Genie). Seventy-three exon candidates suggested by both programs also show high GC levels (usually 60% or greater), an additional indicator of coding capacity. Additional analyses have shown 11 CpG islands, invariable telltales for genes; and the pattern of exons, EST hits, and potential poly(A) addition sites reinforce the inference of a minimum of 11 putative genes in the BAC. This would yield an average of at least one gene every 15 kb, making this region of the t-complex part of the highest gene density fraction (2 to 5%) of the genome.

Tables of the EST hits, candidate genes, and verified genes will be provided. Elsewhere in these Abstracts are reported wet-lab analyses of three genes detected in a 40 kb sample region of the BAC. They include the known gene for Als (which binds and stabilizes the IGF/IGFBP3 complex in serum to extend its half-life), and two newly discovered genes, one of which (Nbps or D17Wsu11e: Nucleotide binding protein short form) shows significant sequence similarity to human NBP as well as yeast and *C. elegans* forms (Grahovac et al., these Abstracts). Availability of the sequence has permitted additional comparisons of predicted and observed exon/intron structures and methylation sites (Shimada et al., these Abstracts).

A31. GENOME ANALYSIS OF THE DISTAL IMPRINTED REGION OF MOUSE CHROMOSOME 7: COMPARATIVE SEQUENCING BETWEEN HUMAN AND MOUSE IDENTIFIES MULTIPLE TISSUE-SPECIFIC ENHANCERS IN THE DOWNSTREAM REGION OF H19.

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Human chromosome band 11p15.5 is implicated in Beckwith-Wiedemann syndrome and childhood tumors, and contains at least seven imprinted genes. The clustering of the imprinted genes suggests that they may be coordinately regulated by a long-range mechanism(s). To complement the genome analysis in human, and to know the mechanism of imprinting regulation of this region, we analyze the syntenic region in mouse chromosome band 7F4/F5. Contigs of YACs, BACs and cosimds which cover approximately 1 Mb were constructed and these will be used for sequencing, gene identification and functional studies in transgenic mice. We also determined a 40-kb sequence of the H19 region in both mouse and human. A comparison of the mouse and human sequences revealed ten small regions with high sequence similarity, two of which precisely coincided with the known enhancers. By producing transgenic mice with reporter constructs, we found that four others exhibit enhancer activity in specific tissues of developing embryos. This enhancer cluster appears to be crucial for the temporal, spatial and imprinted expression of not only *H19*, but also *Igf2*, which is located 100 kb away. Our results demonstrate that comparative sequencing is a straightforward and powerful approach towards the identification of long-acting regulatory elements.

A32. A NEW RAT MUTATION WITH CEREBELLAR VERMIS DEFECT (cvd) MAPS TO CHROMOSOME 2.

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Hereditary cerebellar vermis defect (*cvd*) is a spontaneous autosomal recessive mutation which arose in a stock of the LEW rat strain. Homozygous (*cvd/cvd*) rats display cerebellar vermis defect, fused cerebellar hemispheres, and cerebellar dysplasia. The mutatant rats were crossed once to the Donryu rats, and then inbreeding was started. A strategy for clarifying the pathogenesis of the defects is positional cloning of the causative gene and the present linkage study was carried out as the first step in this respect. Ninety-six backcross progeny from a (ACI x CVD) F1 x CVD cross and 57 backcross progeny from a (F344 x CVD) F1 x CVD cross were raised. The genotype for cvd mutation was determined by the presence or absence of cerebellar vermis and histopathology. In the ACI cross, 43 rats lacked cerebellar vermis (*cvd* type) and the remaining 53 rats were of normal (F1 type). In the F344 cross, 24 rats were *cvd* type and the remaining 33 were F1 type. Out of 46 polymorphic microsatellite markers, 6 markers (*D2Mgh12, D2Rat86, D2Mit15, D2Rat185, D2Rat66, D2Mgh13*) gave highly significant evidence for linkage. As a result, we have mapped a new neurological rat mutation *cvd* on Chr 2, between the *D2Rat185* and the *D2Rat66* locus. To compare the homologous region of the mouse and human, the comparative genetic mapping is underway.

A33. COMPARISON OF THE GENE STRUCTURE OF THE MOUSE AND HUMAN NEURONAL β2-NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) GENES AND PHYSICAL MAPPING OF THE HUMAN GENE.

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The effects of nicotine are mediated by neuronal nAChRs, which are ligand-gated ion channels that respond to the neurotransmitter acetylcholine. Thirteen subunit genes have been identified, and cDNAs for all of the subunits have been cloned, but the gene structure has not been determined for any of them. β2-nAChR forms heterodimers with a number of the other subunits, and is a participant in over 90% of the high affinity nicotine-binding sites in the brain. We have isolated the β2-nAChR genes from mouse and human BAC genomic libraries using a human cDNA as probe, and determined the gene structures. The genes have 6 exons and 5 introns, and while the mouse and human exon/intron boundaries are conserved, the introns all differ in size. Three of the four transmembrane domains are encoded in a single exon. Exon sequences are highly conserved between mouse and human, but the promoters share less than 70% homology. Both promoters have a non-classical TATA, a high GC content (60-80%), and share a number of transcription binding sites. Intron 3 in both species contains a repetitive sequence, a MIR repeat in human and B1 in mouse. The mouse gene, *Acrb2*, had previously been mapped to Chr3 (41.8 cM) in a region syntenic to human Chr1. We mapped the human gene using a 6 Mb YAC contig of Chr 1 that had been isolated from the CEPH mega-YAC library. *CHRNB2* maps to 1q21.3 with the order of markers 1cen, FLG, LOR, S100A6, CHRNB2, 1tel. The order of the mouse genes on Chr3 is cen, *Acrb2, Lor, Flg, Cacy (S100A6)*, tel. Thus, our mapping indicates that an inversion of markers on mouse Chr3 exists with respect to syntenic markers on human Chr1.

A34. MAPPING OF THE TYPE 1 DIABETES LOCUS, *Idd3*, TO A 780 Kb INTERVAL ON PROXIMAL MOUSE CHROMOSOME 3.

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Type 1 diabetes is a complex, immune-based disease that begins with altered self-tolerance and culminates with the destruction of the insulin-producing beta cells of the pancreas. The development of type 1 diabetes in the non-obese diabetic (NOD) mouse has been extensively used as a model for the human disease. Initial studies, using MHC congenic strains, showed that the unique NOD MHC is necessary, but not sufficient, for disease development. Subsequent linkage analyses have demonstrated that at least 16 non-MHC linked loci (*Idd* loci) contribute to disease development in the NOD mouse. Since NOD homozygosity at any one locus does not necessarily lead to the development of diabetes, the mapping of *Idd* loci using conventional outcross strategies is impractical. Consequently, we have adopted a congenic strategy to map individual *Idd* loci. Using a series of congenic strains we have previously mapped *Idd3* to a 0.35 cM interval on proximal mouse chromosome 3. In the present study, through the development of new congenic strains together with the isolation of novel microsatellite markers from a BAC contig constructed across the region we have refined the mapping of *Idd3* to a 780 Kb interval. One gene known to map to this interval is that encoding the cytokine interleukin 2 (IL2). The Il2 gene is a provocative candidate for *Idd3* since mice rendered insensitive to the action of IL2, either through the targeted disruption of the Il2 gene itself or the genes encoding the alpha or beta subunits of its receptor, develop autoimmune disease. We have previously shown that sequence variants exist between IL2 allotypes from different strains of mice and that these variants segregate with disease. SDS-PAGE and Western blot analysis of the NOD and B6 IL2 allotypes revealed that they display different migration profiles. The B6 allotype shows a heterogeneous migration profile with a major band that co-migrates with unglycosylated IL2 and a number of slower migrating glycosylated bands. The NOD allotype has a more homogeneous migration profile made up almost entirely of a single glycoslyated band. Extension of this analysis to IL2 allotypes from different strains showed that mice carrying a diabetes susceptibility allele at *Idd3* displayed the NOD-like, homogeneous migration profile, while those carrying a resistance allele at *Idd3* displayed the heterogeneous migration profile. Moreover, the heterogeneous profile was associated with the presence of serine at position 6 of the mature IL2 protein whereas the homogeneous profile was associated with the presence of proline at position 6. While differential glycosylation appears to have no effect on the ability of different IL2 allotypes to stimulate T cell proliferation, it may alter their ability to deliver the apoptotic signals required to terminate T cell activation and maintain self-tolerance.

A35. GENETIC AND PHYSICAL MAPPING OF *loop-tail*, A MOUSE MUTANT WITH A SEVERE NEURAL TUBE DEFECT, AND ANALYSIS OF CANDIDATE GENES.

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The *loop-tail* (*Lp*) mutant exhibits the most severe form of neural tube defect. Homozygous embryos fail to initiate closure of the neural tube at E8.5, consequently exhibiting an open neural tube from the midbrain throughout the length of the spine. Heterozyous mutants exhibit a tail defect but are viable and fertile. We have used an intraspecific backcross to map the *Lp* gene, and have localised it to a 1.2 cM interval on distal Chromosome 1. A YAC and P1 contig encompassing the closest flanking markers (*D1Mit113* proximally and *Fcer1a* distally) has recently been completed.

This region of distal mouse Chromosome 1 has homology to human 1q21-q23, and examination of the Whitehead and Unigene EST maps revealed several human ESTs that mapped in or near this region. A search of the EMBL database with these human ESTs identified homologous mouse clones, to which PCR primers were designed to allow them to be mapped in our contig. Numerous candidates were localised within the contig, but just outside the critical region, and have therefore been excluded from further study. Several candidates were localised within the critical region, and these genes have been the subject of sequence and expression analysis to assess them as candidates for *Lp*.

Sequence polymorphisms enabled two candidates (*Sm22ah* and *Mor23*) to be excluded as *Lp*, since they show genetic recombination in the backcross panel. Two further candidates (*D1Ucla4* and *Girk3*) have been excluded on the basis that they are not expressed at the time of neurulation. A further five candidates (*Hepcop*, *Pea15*, W66916, AA033172 and *Nhlh1*) are still undergoing analysis, but have so far not yielded any expression or sequence abnormalities in the mutant, and therefore seem unlikely to be responsible for the *Lp* mutation.

A36. A HIGH RESOLUTION GENETIC AND PHYSICAL MAP OF *mdfw*.

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The deaf waddler (*dfw*) mouse mutation was established as a model to study hereditary hearing loss in combination with vestibular dysfunction[1]. Homozygous mutants exhibit significantly elevated thresholds to up to 100dB SPL indicating a profound hearing loss and recently mutations in the plasma membrane bound Ca^{2+} ATPase 2 gene (*Pmca2*) were found to cause these sensory deficits[2,3]. Pmca2 is localized in stereocilia of hair cells and regulates Ca^{2+} homeostasis, neurotransmitter release and Ca^{2+} currents[4,5]. While a glycine to serine substitution in the *dfw* allele presumably reduces ATPase activity and Ca^{2+} translocation in auditory and vestibular hair cells, the 2bp deletion in the dfw^{2J} strain constitutes a functional null mutation.

The modifier-of-deaf waddler locus (*mdfw*) was first recognized in F2 progeny of a CBy/CAST-dfw^{2J} intercross as a recessive BALB/cByJ derived allele ($mdfw^{C}$) that confers progressive hearing loss to ~25% of CBy/CAST-dfw^{2J}/+ F2 heterozygotes. Neither dfw^{2J}/+ heterozygotes nor $mdfw^{C}$ homozygotes independent of each other develop hearing loss suggesting that both loci form a synergistic interaction.

mdfw was initially mapped to Chromosome 10 in close proximity to waltzer (*v*). The closest recombinant flanking markers *D10Mit127* and *D10Mit185* defined a genetic interval of 4cM[2]. Here we report the generation of a high resolution genetic and physical map at *mdfw*.

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A37. GENETIC MAPPING AND MODIFIERS OF THE *bronx waltzer* MUTATION.

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The *bronx waltzer (bv)* mutation is an autosomal recessive mutation mapping to mouse chromosome 5. The receptor cells in the organ of Corti, maculae and cristae are severely affected in homozygous mutant mice. Selective degeneration of inner hair cells in the organ of Corti at gestation day 17 results in the hearing impairment observed in these mutants. The effects of the mutation in the organ of balance (vestibular system) lead to the head bobbing, hyperactivity and circling movements observed in the mice affected by the mutation. Data from intra and interspecific backcrosses allowed the generation of a high resolution map around the bv region. The genotyping of mice originating from the backcross [(*bv*/bv x101/H) F1 x *bv/bv*] resulted in the mapping of *bv* 0.14cM distal to D5Mit 209 and 1.114cM proximal of D5Mit188. The EUCIB, BSS and BSB backcross were also used to check the location of the markers mapped through the bv backcross and to locate other markers and genes in the *bv* region.

When we bred an interspecific backcross between bronx waltzer from the original stock and Mus castaneus we detected positive hearing responses and less severe behavioural abnormalities in some homozygous mutant mice. Behavioural tests were performed to assess the severity of the abnormality and to investigate the possibilty that modifiers of the bv mutation had been introduced from the Mus castaneus genome.

This work was supported by the MRC and Sub-Programa Ciência e Tecnologia do 2º Quadro Comunitario de Apoio.

A38. SYNTENIC ORGANISATION OF THE MOUSE DISTAL CHROMOSOME 7 IMPRINTING CLUSTER AND THE BECKWITH-WIEDEMANN SYNDROME REGION AT CHR. 11p15.5.

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In human and mouse most imprinted genes are arranged in chromosomal clusters. Their linked organisation suggests coordinated mechanisms controlling imprinting and gene expression. The identification of local and regional elements responsible for the epigenetic control of imprinted gene expression will be important to understand the molecular basis of diseases associated with imprinting such as the Beckwith-Wiedemann syndrome. We have established a complete contig of clones along the murine imprinting cluster on distal chromosome 7 syntenic with the human imprinting region at 11p15.5 associated with the Beckwith-Wiedemann syndrome. The cluster comprises about one megabase of DNA, contains at least eight imprinted genes, and is demarcated by the two maternally expressed genes *Tssc3* (*Ipl*) and *H19* which are directly flanked by the non imprinted genes *Nap1l4* (*Nap2*) and *Rpl23l* (*L23mrp*), respectively. We also localised *Kcnq1* (*Kvlqt1*) and *Cd81* (*Tapa-1*) between *Cdkn1c* ($p57^{Kip2}$) and *Mash2*. The mouse *Kcnq1* gene is maternally expressed in most foetal but biallelically transcribed in most neonatal tissues suggesting relaxation of imprinting during development. Our findings indicate conserved control mechanisms between mouse and human, but also reveal some structural and functional differences. Our study opens the way for a systematic analysis of the cluster by genetic manipulation in the mouse which will lead to animal models of Beckwith-Wiedemann syndrome and childhood tumors.

A39. A COMBINED GENETIC AND PHYSICAL MAP OF DISTAL MOUSE CHROMOSOME 12.

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We have identified a 4 Mb YAC contig containing the *Igh* locus and adjoining genes on distal chromosome 12, and have partially replicated this with a 1 Mb BAC contig. The contigs contain the proximal boundary of the evolutionary diverse *Igh* locus, but we have not located the distal boundary or the telomere. We are integrating the physical map, the genetic map, the B cell deletion map and the Radiation Hybrid map.

A40. MOLECULAR CHARACTERIZATION OF 10 GENES MAPPED ON THE MOUSE t-COMPLEX.

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While mapping genes recovered from 7.5-dpc mouse ectoplacental cone cDNA library, we found 10 genes clustered in the t-complex on Chromosome 17. To gain insight into the nature of this gene cluster, we started a detailed molecular analysis of individual genes. *D17Wsu155e, D17Wsu19e,* and *D17Wsu134e* were clustered in the same bin as *Plg*. Detailed sequence analysis revealed that *D17Wsu155e* encodes a mouse homolog of human RSK3, pp90rsk Ser/Thr kinase. *D17Wsu19e* is a mouse alpha-tubulin isotype M-alpha-2. *D17Wsu134e* encodes a 3.0 kb transcript, which does not have any similar sequences in the public database.

D17Wsu11e, D17Wsu15e, D17Wsu51e, and *D17Wsu82e* were clustered in the same bin as D17Mit55. D17Wsu11e (Nbps; Grahovac et al., these abstracts) and *D17Wsu15e* were located in a single BAC clone, which has been analyzed extensively (Kargul et al. and Shimada et al. these abstracts). D17Wsu51e encodes a 4.5 kb transcript, which does not show any similarity hits in the database. This gene is expressed most highly at 7-dpc and then its expression decreases. *D17Wsu82e* encodes a 1.35 kb transcript, which shows a strong similarity to the exons recovered from the human Chromosome 16p13.3 and probably codes for a transcription factor.

D17Wsu92e is located between these clusters and also has no apparent hits in the sequence database.

D17Wsu91e and *D17Wsu76e* were clustered in the same bin as *D17Mit16*. *D17Wsu76e* encodes a 4.4 kb transcript, which shows a strong similarity to GEG68 (Sperm/Oocyte expressed gene). However, Northern blot analysis showed rather ubiquitous expression of this gene. *D17Wsu91e* encodes an unknown gene, which shows an expression in the trophoblast giant cells. *D17Wsu166e* mapped to the H2 region and encodes a 3.6 kb transcript. This gene has a motif of the ATP-binding transport protein (ABC transporter family). The expression is very high at 7 dpc and then decreases.

Thus, the genes recovered are relatively specifically expressed at this time and in this region during embryonic development, some of them at very high levels. Many of these are candidates for involvement in transcriptional and structural changes during the nascent formation of the placenta and associated tissues; and it may therefore not be surprising that they are clustered in the t-complex, where a number of mutations result in embryonic lethality. These genes are being mapped relative to t-mutants using DNA from a series of partial t-haplotypes that contain different portions of the t chromosome. For example, Southern blot analyses have mapped *D17Wsu11e* and *D17Wsu51e* based on RFLPs between C3H.Brtf and tw5G/tw5G. These mapping studies will help to determine cDNAs that might warrant priority analysis for t-complex lethal mutants and corresponding critical roles in embryonic development.

A41. A 600 kb BAC CONTIG COMPRISING THE LOCI wobbler, Mor2, Otx1, TYPE IIB IAP INTEGRASE AND FIVE NEW TESTIS EXPRESSED GENES ON MOUSE CHROMOSOME 11.

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We are engaged in the positional cloning of the wobbler (*wr*) gene on mouse Chr 11 (Kaupmann et al., 1992), the recessive defective allele of which causes a spinal muscular atrophy and a spermiogenesis defect. Combining physical and genetical mapping data the candidate interval has been narrowed down to approximately 600 kb covered by seven overlapping BACs (Resch et al., 1998). To identify positional candidates direct cDNA-selection (Lovett et al., 1991) was used. The *wr* gene should be expressed in the CNS and testis as inferred from *wr/wr*<<+/+ chimeric mice (Augustin et al., 1997). Hybridisation was performed with a cDNA-library of wildtype testis and pools of BACs covering the *wr* critical region. Six selected cDNA fragments were cloned, sequenced and mapped. One fragment showed a 99% sequence homology to a type IIB intracisternal A-particle elements encoding integrase. No significant homology for five other cDNA fragments was found. Tissue distribution of these cDNA clones were confirmed by RT-PCR.These novel testis expressed genes are being tested as candidates for the wobbler disease gene by mutation analysis.

Supported by Deutsche Forschungsgemeinschaft SCHM 1276.

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A42. EXPLOITING UNAFFECTED F2 AND F3 LITTERMATES OF HOMOZYGOUS MUTANTS WITH INFORMATIVE RECOMBINATION BREAKPOINTS: A SIMPLE STRATEGY TO AVOID LARGE BACKCROSS/INTERCROSS PROGENY AS APPLIED TO THE MOUSE MUTATION wobbler.

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In the course of the positional cloning approach for the identification of the recessive wobbler (wr) mutation of the mouse, which causes a spinal muscular atrophy and a spermiogenesis defect, the wr locus was located to a candidate interval, genetically defined by inter- and intra-strain backcross systems (Kaupmann et al., 1992; Korthaus et al., 1996). This candidate interval of about 1 cM was extensively studied by physical mapping (Wedemeyer et al., 1996; Resch et al., 1998). One important goal of the positional cloning project is to narrow down the candidate interval for to decrease the number of candidate genes. For this purpose usually thousands of backcross animals have to be analysed. Here we report on a simple strategy using F2 and F3 crosses, which allows to identify informative recombination events in affected animals as well as unaffected littermates. The analysis of all offspring is possible not only of the Mendelian quarter as is the case in backcross systems. This strategy led to the identification of informative recombinations which redefine the candidate region for the wr gene.

Supported by Deutsche Forschungsgemeinschaft SCHM 1276.

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A43. POLYMORPHISMS AT THE CCT4 LOCUS ON MOUSE CHROMOSOME 11: APPLIED FOR A INTRA-STRAIN DIAGNOSIS OF THE wobbler (wr) GENE.

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The loci *Cct4* and wobbler (wr) map very close to each other (0.6 cM) on proximal mouse Chr 11. The *Cct4* gene encodes the delta subunit of the cytosolic chaperonin complex containing the t-complex polypeptide 1 (TCP-1). Homozygous *wr/wr* mice have been used as phenotypic models for spinal muscular atrophies, i.e. muscle wasting due to neurodegeneration, and for male sterility due to a defect in spermiogenesis. Here we report on the discovery of multiple polymorphisms of the *Cct4* gene which is closely linked to the *wr* gene (Schmidt et al., 1998). Therefore *Cct4* appeared to be a good candidate for the *wr* gene but *Cct4* was excluded on the basis of its location on the physical map (Wedemeyer et al., 1996; Resch et al., 1998) of the wobbler region. Furthermore, a recombination between *Cct4* and *wr* was identified. Novel insights into the pathomechanisms of the neurodegeneration and male sterility require approaches by experimental embryology and studies on early phases of development, and therefore an early diagnosis of *wr/wr* individuals. These *Cct4* polymorphisms have obviously arisen within C57BL/6J strain thus allowing for a diagnosis of the allelic status at the wr locus without the disadvantages of interstrain crosses. Furthermore, we describe the insertion of a 210 bp B2 repetitive element into *Cct4* gene of the AKR strain (Schmidt et al., 1998).

Supported by Deutsche Forschungsgemeinschaft SCHM 1276.

Resch, K., Korthaus, D., Wedemeyer,N., Lengeling, Thiel, C., Ronsiek, M., Baer, K., Jockusch, H. and Schmitt-John, T. (1998) Homology between human Chr 2p13 and the wobbler critical region on mouse Chr 11: Comparative high resolution mapping of STS and EST loci on YAC/BAC contigs Mamm Genome (in press).

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A44. LINKAGE ANALYSIS OF THE REPEATED EPILATION (*Er*) REGION ON MOUSE CHROMOSOME 4 REVEALS SEX AND STRAIN-SPECIFIC DIFFERENCES IN RECOMBINATION FREQUENCIES.

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The skin provides an organism with the first line of defense against external factors. Abnormal development of the highly organized epidermal layers and numerous appendages, such as the hair follicles, leave the organism vulnerable to harsh environments. The Repeated epilation (*Er*) mutant mouse provides a model system to dissect molecular pathways involved in skin development and tumorigenesis. Mice homozygous for the mutation die postnatally by asphyxiation and display disturbances in epidermal organization along with morphological disruptions in growth and development of the limbs, tail, and facies. *Er/+* mice display a unique phenotype of repeated hair loss and regrowth throughout life with little apparent morphological disruption of the epidermis. In addition, adult *Er/+* mice have an increased risk of cutaneous papillomas and squamous cell carcinomas. To positionally clone the gene(s) responsible for the *Er* mutation, we established an intersubspecific backcross to Mus musculus castaneus and an interspecific backcross to *Mus spretus*. We now report genotyping results that include more than one dozen genes and molecular markers in >1,000 animals. The results refine the chromosome 4 region that contains *Er* and eliminate several genes as candidates for the *Er* mutation. Haplotype analysis revealed statistically significant evidence for sex-specific as well as strain-specific differences in recombination frequencies. Additional characterization of aged *Er/+* mice confirm and extend previously reported findings regarding the incidence and pathology of skin lesions associated with the mutant *Er* allele. Research supported in part by ACS grant PF-4230 to RAL and NCI grant CA21124 to LDS.

A45. MAPPING OF A LUNG TUMOR SUSCEPTIBILITY GENE WITHIN THE CLASS III REGION OF THE MOUSE MHC.

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Two intra-H2 recombinant mouse strains, B10.A(1R) and B10.A(2R) differ in their susceptibility for chemically induced alveolair lung tumors. We analysed the strains for their MHC profiles, using RFLP and microsatellite typing. Both strains have a cross-over in the *Hsp70-G7* interval in the class III region of the MHC. Cloning of the region and generating of new microsatellite markers revealed a hotspot of recombination in which the breakpoint of one of the two strains was localised, the other is still to be found. The maximal length in which these two strains might differ is 27 kb. This interval contains three genes, *G7e, G7a* and *G7c,* one of which should be involved in the control of the susceptibility to lung tumors.

G7e shows similarity to viral envelop encoding genes, *G7a* encodes valyl-tRNA synthetase, and *G7c* is a novel gene with unknown function. We plan to sequence the entire interval of both recombinant haplotypes in order to find clues, which of the genes is involved. The ultimate proof for a gene to be the susceptibility gene has to come from a transgenic or knockin setting.

A46. A PHYSICAL MAP OF THE MOUSE X CHROMOSOME.

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We have assembled a genetically-anchored YAC STS content map of the mouse X chromosome using semi-automated PCR screening of the large-insert Whitehead/MIT YAC library (average insert size 820kb). The map landmarks are a combination of STSs developed here at the Mouse Genome Centre, SSLP markers from the Whitehead/MIT Genome Centre and X-linked genes. Chromosome specific libraries were constructed from material enriched by flow-sorting and end-sequencing of clones from these libraries allowed us to develop 1056 STSs. Genetic mapping of a subset of these STSs showed that greater than 90% mapped back to the X chromosome. As well as demonstrating the high purity of the flow-sorted material, genetic mapping has improved the anchoring of the STS content YAC map. We have added more than 80 flow-sort STSs to the X chromosome, using SSCP to genotype animals from the high resolution EuropeanBackcross (EUCIB) resource.

PCR screening and STS generation are supported by a lab data management system, "HOSEpipe" (High Output STS Evaluation pipeline; Strivens et al (1997)). This is based around a local WWW server and uses new and existing tools to design STSs and improve the flow and accuracy of STS data recording.

In total, approximately 4,400 YAC co-ordinates have been identified for 840 STS. To date, 611 markers have been incorporated into the STS-content physical map of the mouse X chromosome giving a density of about 1 marker per 250 kb. The final map release will be in August 1998 and will be accessible at the URL: http://www.mgc.har.mrc.ac.uk/xmap/xmap.html

A47. GENETIC ANALYSIS FOR SPONTANEOUS TONIC-CLONIC SEIZURES IN NER RAT STRAIN.

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We have developed a novel epileptic rat strain NER (Noda Epileptic Rat) by inbreeding rats with spontaneous tonic-clonic seizures in a stock of Crj:Wistar (Noda et al., Epilepsia, 39(1):99-107, 1998). Most seizure onsets occurred between 8-16 weeks of age, and the incidence was approximately once in 30 hours. Ictal cortical and hippocampal electroencephalograms were characterized by high-voltage spikes followed by diffuse spike-and-wave or polyspike-and-wave complexes. NER, with no distinct brain lesions, revealed seizure susceptibility to pentylenetetrazol, tossing, and transcorneal electroshock. To analyze the mode of inheritance of spontaneous tonic-clonic seizures and to map genes contributing to the seizure susceptibility, we produced (F344/DuCrj x NER)F1 hybrids and (F344/DuCrj x NER)F1 x NER backcross progeny. None of the 9 F1 hybrids and 10 F344/DuCrj rats exhibited tonic-clonic seizures by age 48 weeks. Out of a total of 204 backcross progeny, 132 (64.7%) showed tonic-clonic seizures by the age. Such seizures were observed from 20 weeks of age in 2 rats, and the accumulated number of rats showing seizures increased gradually until 48 weeks of age. These results suggest that the seizure of NER may be controlled by a major autosomal recessive gene and several modifier genes. Whole genome scanning on the backcross progeny is currently underway. When an early onset group, in which tonic-clonic seizures were observed from age 20-26 weeks, was collected and analyzed, a notable association with the seizure was found on chromosome 3.

A48. ANALYSIS OF SYNTENIC RELATIONSHIP BETWEEN MOUSE CHROMOSOME 9 AND HUMAN CHROMOSOME 6 BY RADIATION HYBRID MAPPING.

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Comparative genome studies have shown presence of a conserved gene order between different species within chromosomal segments, which is called synteny. A quality (in accuracy, resolution, and comprehensiveness) of a synteny analysis depends on a number of orthologous gene loci included and also on mapping methods. In recent years, availability of sequence information of genes has dramatically increased in human and mouse as a result of on-going EST projects. Furthermore, establishment of human and mouse radiation hybrid (RH) panels has greatly expanded our ability to map a large number of genes on single panels relatively easily and quickly with a high resolution.

We are taking an advantage of RH mapping techniques to analyze syntenic relationship between mouse chromosome 9 (MMU9) and human chromosome 6 (HSA6). Earlier studies have indicated that a middle segment of MMU9 (Map Position 42-48) and a region encompassing the centromere of HSA6 (6p21-6q15) are syntenic. These chromosomal segments contain several of cloned gene loci including *Bmp5*/BMP5, *Col12a1*/COL12A1, *Myo6*/MYO6, *Htr1b*/HTR1B, and *Mod1*/ME1. Since none of the previous studies included all of these loci for mapping in a single panel and some genes were mapped only by a low resolution mapping method (FISH or somatic cell hybrid mapping), synteny between MMU9 and HSA6 is not defined in detail.

We will show RH mapping data from two human panels (GB4 and G3) and a mouse panel (T31) in comparison. We are mapping orthologous genes (or ESTs) in a systematic way to establish a high-density synteny map, and we are also attempting to narrow down break-points of the syntenic segments between MMU9 and HSA6.

A49. MOLECULAR AND GENETIC ANALYSIS OF LURCHER (*Lc*) AND PURKINJE CELL DEGENERATION (*pcd*) MUTATIONS.

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Naturally occurring mutant mice, Lurcher (*Lc*/+) and Purkinje cell degeneration (*pcd/pcd*), display cerebellar ataxia and other neurologic symptoms. The common cellular targets of these two mutations are the cerebellar Purkinje cells (PC), which degenerate during the first few months after birth. While the effect of the *Lc* mutation is restricted to PC, the *pcd* mutation targets on several other CNS neuronal cell types, such as photoreceptor cells in retina, mitral cells in olfactory bulb, and neurons in thalamus.

To understand the mechanisms of neurodegeneration in *Lc*/+ and *pcd/pcd* mice, we adopted a positional cloning approach to identifying the mutant genes responsible for the *Lc* and *pcd* phenotypes. For the semi-dominant mutation Lc, we identified a missense mutation in a highly conserved transmembrane domain of the glutamate receptor delta 2 gene; this mutation causes the channel to be constitutively open, leading to the apoptotic death of PC (Nature, 388: 769). This gain-of-function mutation is different from the loss-of-function alleles of the same gene in knockout mice.

For the recessive mutation *pcd*, we have examined four different mutant alleles, *pcd*, *pcd**2J, *pcd**3J and pcd*sid. They all display similar but slightly different phenotypes and the latter three mutations failed to complement the original *pcd* allele. To map the mutations more precisely, we have generated several intrasubspecific crosses between *pcd* strains and CAST/Ei and MOLG/Dn. By analyzing 117 meioses in a pcd/CAST backcross, and 70 meioses in a *pcd**3J/CAST intercross, we located the *pcd* mutations within a 1.6 cM region on mouse Chromosome 13. This region is within the 5.6 cM congenic region of *pcd* analyzed by others, thus validating our mapping results. This 1.6 cM region is mostly covered by a single Whitehead YAC contig, and it is syntenic to a well-characterized region in the human genome. Currently, we are in the process of identifying cDNA clones and establishing a BAC contig of the *pcd* region.

This work was supported in part by NIH Cancer Center Support CORE grant P30 CA21765 and by the American Lebanese Syrian Associated Charities (ALSAC).

B. Gene Identification & Mutation Analysis

Abstracts - Presentations

B1. ANALYSIS OF SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) IN THE MOUSE.

David R. Beier, *Isaac M. Neuhaus. Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.*

B2. LOSS OF HETEROZYGOSITY AT THE dilute - short ear REGION OF THE MOUSE: MITOTIC RECOMBINATION OR DOUBLE NON-DISJUNCTION?

Jack Favor, *Angelika Neuhäuser-Klaus. Institute of Mammalian Genetics, GSF-National Research Center for Environment and Health, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.*

B3. *nervous* ENCODES A NOVEL PROTEIN WITH LIMITED HOMOLOGY TO PROTEINS INVOLVED IN LIPID METABOLISM.

Colin F. Fletcher, *Linda S. Cleveland, Neal G. Copeland, Nancy A. Jenkins. Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD, USA 21702.*

B4. THE *itchy* LOCUS ENCODES A NOVEL UBIQUITIN PROTEIN LIGASE THAT IS DISRUPTED IN a^{18H} MICE.

William L. Perry[1,2], Carolyn M. Hustad[1,3], Deborah A. Swing[1], T. Norene O'Sullivan[1], Nancy A. Jenkins[1], **Neal G. Copeland[1].** *[1]Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD, USA 21702; [2]Present address: Eli Lilly and Company, Indianapolis, IN, USA 46285; [3]Present address: Zeneca Pharmaceuticals, Wilmington, DE, USA 19850.*

B5. MUTATIONS IN Pmca2 UNDERLY THE deafwaddler and deafwaddler 2J PHENOTYPES.

Jennifer (Jenna) McKee-Johnson, *UW School of Medicine.*

B6. POSITIONAL CLONING OF THE MOUSE NEUROLOGICAL MUTANT ducky (du).

Jane Barclay[1], *Kenro Kusumi[2], Magali Williamson[1], Eric Lander[2], Edward Perez-Reyes[3], Wayne Frankel[4], Mark Gardiner[1], Michele Rees[1]. [1]Department of Paediatrics, University College London, WC1E 6JJ, UK; [2]Whitehead Institute, Nine Cambridge Center, Cambridge, MA 02142-1479, USA; [3]Department of Physiology, Loyola University Medical Center, Maywood, IL 60153, USA; [4]The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

B7. A VERY LARGE NOVEL PROTEIN WITH DIVERSE FUNCTIONAL MOTIFS IS DEFICIENT IN *rjs* (RUNTY, JERKY, STERILE) MICE.

Anne L. Lehman[1], Yoshimichi Nakatsu[1], Ada Ching[1], Roderick T. Bronson[2], Rebecca J. Oakey[1], Natalie Keiper-Hrynko[1], Joshua N. Finger[1], Donna Durham-Pierre[1], Daniel B. Horton[1], J. Michael Newton[3], Mary F. Lyon[4], **Murray H. Brilliant[1,3].** *[1]Formerly at Fox Chase Cancer Center, Philadelphia, PA, [2]Jackson Laboratory, Bar Harbor, ME, [3]University of Arizona School of Medicine, Tucson, AZ, [4]MRC, Harwell, UK.*

B8. MOLECULAR ANALYSIS OF MOUSE NEURAL CREST DEVELOPMENT AND DISEASE USING cDNA EXPRESSION PROFILE ANALYSIS.

[1]U Teichmann, *[2]M Bittner, [3]G Schuler, [1]T Otsuka, [4]G Merlino, [5]T Moore, [2]Y Chen, [2]P Meltzer, [2]J Trent, [1]W Pavan. [1]Genetic Disease Research Branch, [2]Cancer Genetics Branch, National Human Genome Research Institute, [4]Laboratory of Molecular Biology, National Cancer Institute, [3]National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD 20892, [5]Research Genetics, Huntsville, AL 35801.*

B9. IDENTIFICATION OF PROTEINS THAT INTERACT WITH THE BEIGE (CHEDIAK-HIGASHI SYNDROME) PROTEIN (LYST), USING A YEAST TWO-HYBRID METHOD.

Velizar T. Tchernev, *Krishnan Nandabalan*, Madan Kumar*, Vishnu S. Mishra, Stephen F. Kingsmore*. Departments of Medicine and Pathology, University of Florida; *CuraGen Corporation, New Haven, CT.*

B. Gene Identification & Mutation Analysis

B1. ANALYSIS OF SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) IN THE MOUSE.

David R. Beier, *Isaac M. Neuhaus. Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.*

Genetic analysis in inbred mice is efficient because haplotypes are fixed and the phase of alleles in a cross are known. In contrast, linkage analysis of human populations is more difficult, because linkage disequilibrium is maintained over relatively short intervals (100 kb or less). This is particularly problematic for complex traits, in which a locus contributing a portion of phenotypic variance can often only be detected by an association study using very tightly linked markers. To address this, investigators of human diseases have recognized that a much more dense genetic map is required, and a highly efficient (and preferably automated) means for genotype analysis is necessary. Microsatellite markers containing simple sequence length repeats do not fulfill this need, since they are insufficiently abundant and their analysis requires gels. As an alternative, there is considerable enthusiasm for (and investment in) the identification of single nucleotide polymorphisms (SNPs) as a bi-allelic system. While the informativeness of these markers individually is less than SSLP markers, this is offset by their frequency, which empirical studies suggest are at least one per 1000 bp, and the potential for high-throughput analysis using techniques such as hybridization to DNA "chips".

While SSLP markers can be used effectively for genetic studies in the mouse, their analysis can be tedious and inefficient. This is particularly true when using a strain for which the allelic variants are not known, in which case the informativeness of each marker must be tested. Thus a means to perform automated genotyping in mouse genetic studies is desirable. Given that a substantial effort is in progress to develop this technology for human genetic studies, we propose that the characterization of SNPs for murine studies would be useful. Additionally, an efficient method of detecting single nucleotide variants would have obvious utility as a means to both assess the efficiency of ENU mutagenesis and to test for mutations in a specific gene in a mutagenesis experiment.

We have experience in both the analysis of sequence variants in inbred strains and in mutagenesis. In studies utilizing SSCP analysis of 3' UTR as a means for genetic mapping, we have demonstrated that polymorphism between inbred strains is readily detectable and can be used for genetic mapping in RI panels. In an analysis of over 800 primer pairs, the polymorphism frequency was as much as 10-20% of fragments tested for several strain combinations, corresponding to 0.5-1 variants per 1000 bp. We are presently testing this more specifically, as well as examining the polymorphism frequency found in anonymous genomic sequence derived from BAC ends. We have used SSCP analysis for these studies, as well as for assessing mutagenesis efficiency in ENU experiments. We are also testing other methods of SNP detection, such as MutS binding, DHPLC, and direct sequencing. The development of even moderately densely distributed SNPs will facilitate rapid and cost-effective genome-wide linkage analysis.

B2. LOSS OF HETEROZYGOSITY AT THE dilute - short ear REGION OF THE MOUSE: MITOTIC RECOMBINATION OR DOUBLE NON-DISJUNCTION?

Jack Favor, *Angelika Neuhäuser-Klaus. Institute of Mammalian Genetics, GSF-National Research Center for Environment and Health, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.*

The specific locus mutation test of the mouse was developed to efficiently screen for germ line mutations at 7 autosomal loci distributed among 5 chromosomes. The vast array of mutant alleles generated in such experiments have been valuable for studies on the nature of induced mutations and the organization of the mouse genome. The tightly linked Chromosome 9 markers dilute (d = *Myo5a*) and short ear (se = *Bmp5*) have been intensively studied. One class of mutation in the region, d - se double mutants, has been of particular interest. Most have been shown to be due to a deletion involving both loci. However, one group of d - se presumed double mutations was identified to be homozygous viable, indistinguishable from the d - se alleles carried by the Tester-stock and remained elusive to further genetic or molecular analyses. As an initial hypothesis, the mechanism involved in the occurrence of such mutations was assumed to be double non-disjunction such that the original mutant inherited 2 copies of Chromosome 9 from the Tester-stock parent and 0 copies from the homozygous wildtype parent. A number of other mechanisms could also account for the homozygous viable double mutants including simultaneous mutations at both loci, gene conversion or mitotic recombination but methodologies were not in place to analyse and differentiate among the mechanisms when the mutants were recovered. With the recent characterization of the mouse genome for highly polymorphic microsatellite markers this situation has changed and an analysis of newly occurring mutants for flanking genetic markers is now possible and practical. Due to eventual crossing over it was imperative that an original homozygous viable d - se double mutant as well as the immediate segregants from the original mutant be analysed. We were fortunate to have recovered such a mutation recently and we undertook the appropriate analyses. Results indicate the mechanism of occurrence of the double mutant to be mitotic recombination and not double non-disjunction.

Research supported by EEC Contract Number CHRX-CT93-0181.

B3. *nervous* ENCODES A NOVEL PROTEIN WITH LIMITED HOMOLOGY TO PROTEINS INVOLVED IN LIPID METABOLISM.

Colin F. Fletcher, *Linda S. Cleveland, Neal G. Copeland, Nancy A. Jenkins. Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD, USA 21702.*

The *nervous* mutation arose spontaneously in a BALB/cGr subline carrying tk (*tail-kinks*). Homozygotes can be recognized at 2 to 3 weeks of birth by their small body size, hyperactivity, and ataxic gait. Significant pathological features include alterations in the distribution and appearance of mitochondria in all *nervous* Purkinje cells preceding the selective degeneration of some Purkinje cells in the hemispheres and caudal vermis of the cerebellum and a pronounced reaction with the lipid-sensitive stain Oil Red O. Electron microscopy revealed the presence of spherical mitochondria in many neuronal cell types in the week preceding frank degeneration, with surviving neurons eventually reacquiring normal mitochondrial profiles. The Purkinje cell degeneration was subsequently shown to occur in a regular pattern of longitudinal stripes. We have observed that neuronal death occurs between postnatal days 20 and 28, coincident with the segregation of afferent excitatory climbing fiber and local inhibitory synapses. Given that *nervous* could be a model of selective neuronal death with an unusual pathology, we sought to identify the causative mutation and characterize the affected gene. We have supplemented the usual positional cloning strategy with BAC complementation to identify a 50 kb interval that must contain the *nervous* gene. Sequencing of this interval identified a gene encoding a novel protein that harbored a missense mutation in nervous RNA and DNA relative to the strain of origin. We have identified homologous genes in *C. elegans, Drosophila,* and humans. The protein contains similarities to enzymes involved in phosopholipid biosynthesis, leading us to focus our investigations into the mechanism of this unusual pathology on a derangement of lipid metabolism. [Research sponsored by the National Cancer Institute under contract with ABL]

B4. THE *itchy* LOCUS ENCODES A NOVEL UBIQUITIN PROTEIN LIGASE THAT IS DISRUPTED IN a^{18H} MICE.

William L. Perry[1,2], Carolyn M. Hustad[1,3], Deborah A. Swing[1], T. Norene O'Sullivan[1], Nancy A. Jenkins[1], **Neal G. Copeland[1].** *[1]Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD, USA 21702; [2]Present address: Eli Lilly and Company, Indianapolis, IN, USA 46285; [3]Present address: Zeneca Pharmaceuticals, Wilmington, DE, USA 19850.*

Non-agouti-lethal 18H (α^{18H}) mice are dark agouti with black pinna hairs. What makes these mice unique is that they develop a spectrum of immunological diseases not seen in other agouti mutant mice. On the JU/Ct background, α^{18H} mice develop an inflammatory disease of the large intestine. On the C57BL/6J background, they develop a fatal disease characterized by pulmonary chronic interstitial inflammation and aveolar proteionosis, inflammation of the glandular stomach and skin resulting in scarring due to constant itching, and hyperplasia of lymphoid cells, hematopoietic cells and the forestomach epithelium. Previous studies suggested that α^{18H} results from a paracentric inversion that affects two loci: *agouti* and another locus designated *itchy* (*itch*) (Hustad et al. *Genetics* 140: 255-265, 1995). In studies to be described, we will show that α^{18H} does indeed result from an inversion. We will also show that itch encodes a novel E3 ubiquitin protein ligase, a protein involved in ubiquitin-mediated protein degradation. While the protein targets of Itchy have yet to be defined, one clue comes from the phenotypic similarity between α^{18H} mice and *motheaten* (*me*) mice. The *me* locus encodes hematopoietic cell phosphatase (Hcph), which is known to function in a number of cytokine pathways that regulate growth and differentiation of hematopoietic cells. It is conceivable that Hcph is a direct target of Itchy or alternatively that Itchy might regulate one or more cytokine receptors located upstream of Hcph. Our results indicate that ubiquitin-dependent proteolysis is an important mediator of the immune response *in vivo*. Further elucidation of Itchy's role in preventing inflammation could result in the development of drugs that would impact wound-healing and chronic inflammation in humans and, theoretically, may help to relieve the *itch*. [Research sponsored by the National Cancer Institute under contract with ABL]

B5. MUTATIONS IN A PLASMA MEMBRANE Ca2+-ATPase GENE CAUSE DEAFNESS IN deafwaddler MICE.

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Hearing loss is the most common sensory deficit in humans. Because the auditory systems of mice and humans are conserved, studies on mouse models have predicted several human deafness genes as well as identified novel genes involved in hearing1, 2. The deafwaddler (dfw) mouse mutant is deaf and displays vestibular/motor imbalance. Here we report that the plasma membrane calcium-ATPase type 2 gene (Atp2b2, also known as Pmca2) is mutated in dfw. An A-to-G nucleotide transition in dfw DNA causes a glycine to serine substitution at a highly conserved amino acid position, while in a second allele, dfw2J, a 2 base pair deletion causes a frameshift predicting a truncated protein. In the cochlea, ATP2B2 is localized to stereocilia and the basolateral wall of hair cells in wildtype mice but is not detected in dfw2J mice. Thus, mutation of Atp2b2 may cause deafness and imbalance by affecting sensory transduction in stereocilia3 as well as neurotransmitter release from the basolateral membrane4. Our identification of mutations affecting Atp2b2 in dfw and dfw2J provides the first example of mutants in a mammalian plasma membrane calcium pump and defines a new class of deafness genes directly affecting hair cell physiology.

B6. POSITIONAL CLONING OF THE MOUSE NEUROLOGICAL MUTANT ducky (du).

Jane Barclay[1], *Kenro Kusumi*[2], *Magali Williamson*[1], *Eric Lander*[2], *Edward Perez-Reyes*[3], *Wayne Frankel*[4], *Mark Gardiner*[1], *Michele Rees*[1]. [1]*Department of Paediatrics, University College London, WC1E 6JJ, UK;* [2]*Whitehead Institute, Nine Cambridge Center, Cambridge, MA 02142-1479, USA;* [3]*Department of Physiology, Loyola University Medical Center, Maywood, IL 60153, USA;* [4]*The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

ducky (*du*) is a spontaneous autosomal recessive mutation which arose in the non-inbred ruby silver stock of mice. It is thought to be a valid model of human idiopathic generalised epilepsy on the basis of spike wave discharges recorded on EEG examination. Additionally, homozygous ducky mice display ataxia, hind brain dysgenesis and demyelination and axonal dystrophy of selected nerve fibres.

The ducky mutation was mapped to mouse chromosome 9 by linkage to the dilute and short ear loci. Progeny from 2 crosses: a (TKDU-du x STOCK-pu)F1 intercross and a (TKDU-du x *M.m.castaneus*) x TKDU-du backcross have been used to refine the candidate interval to approximately 0.4cM. A physical map of the ducky candidate region was generated in a series of overlapping YAC clones.

Several known genes including *Dag-1*, *Lamb2* and *Col7A1* were excluded as the ducky gene by virtue of their position. However, their proximity to the candidate region indicated that human chromosome 3p21 would contain the human orthologue to the ducky gene. Database searching identified a gene from this area which showed significant homology to a voltage gated calcium channel subunit. The previous publication of two genes encoding voltage dependent calcium channel subunits as underlying the tottering (Fletcher et al., 1996) and lethargic (Burgess et al., 1997) models of spike wave epilepsy made this gene an excellent candidate for the ducky mutation.

The murine orthologue of this novel calcium channel subunit has been shown to map to the ducky candidate region of mouse chromosome 9. Northern analysis and semi-quantitative RT-PCR has demonstrated reduced levels of transcript in homozygous ducky brains compared to controls. This suggests its disruption is responsible for the ducky phenotype. Mutational analysis of the murine homologue in the ducky mouse and current functional characterisation of this novel subunit will be presented.

B7. A VERY LARGE NOVEL PROTEIN WITH DIVERSE FUNCTIONAL MOTIFS IS DEFICIENT IN *rjs* (RUNTY, JERKY, STERILE) MICE.

Anne L. Lehman[1], Yoshimichi Nakatsu[1], Ada Ching[1], Roderick T. Bronson[2], Rebecca J. Oakey[1], Natalie Keiper-Hrynko[1], Joshua N. Finger[1], Donna Durham-Pierre[1], Daniel B. Horton[1], J. Michael Newton[3], Mary F. Lyon[4], **Murray H. Brilliant[1,3].** *[1]Formerly at Fox Chase Cancer Center, Philadelphia, PA, [2]Jackson Laboratory, Bar Harbor, ME, [3]University of Arizona School of Medicine, Tucson, AZ, [4]MRC, Harwell, UK.*

Three radiation-induced alleles of the mouse *p* locus, p^{6H}, p^{25H}, and p^{bs}, cause defects in growth, coordination, fertility and maternal behavior in addition to *p* gene-related hypopigmentation. We have found that these alleles are associated with disruptions of the *p* gene and disruptions in a single adjacent gene involved in the disorders listed. We have identified this adjacent gene, previously named *rjs* (runty jerky sterile), by positional cloning. The *rjs* cDNA is very large, covering 15,264 nucleotides, encoding a predicted protein of 4836 amino acids with a molecular weight of 527 kDa. The *rjs* protein contains several sequence motifs, including three RCC1 repeats, a structural motif in common with cytochrome b5, and a HECT domain in common with E6-AP ubiquitin ligase. Based on sequence homology and conserved synteny, the *rjs* gene is the single mouse homolog of a previously described 5-6 member human gene family. This family is represented by at least two genes, HSC7541 and KIAA0393, from human chromosome 15q11-q13. HSC7541 and KIAA0393 lie close to, or within, a region commonly deleted in most Prader-Willi syndrome patients. Previous work has suggested that the multiple phenotypes in rjs mice might be due to a common neuro-endocrine defect. In addition to this proposed mode of action, alternative functions of the *rjs* gene are possible in light of its known protein homologies.

B8. MOLECULAR ANALYSIS OF MOUSE NEURAL CREST DEVELOPMENT AND DISEASE USING cDNA EXPRESSION PROFILE ANALYSIS.

[1]**U Teichmann**, [2]*M Bittner*, [3]*G Schuler*, [1]*T Otsuka*, [4]*G Merlino*, [5]*T Moore*, [2]*Y Chen*, [2]*P Meltzer*, [2]*J Trent*, [1]*W Pavan*.
[1]*Genetic Disease Research Branch*, [2]*Cancer Genetics Branch, National Human Genome Research Institute*, [4]*Laboratory of Molecular Biology, National Cancer Institute*, [3]*National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD 20892*, [5]*Research Genetics, Huntsville, AL 35801*.

Several mouse mutants exist that display defects in neural crest development. These include lack of specific derivatives, (e.g., spotting mutants) or uncontrolled proliferation (e.g., melanoma susceptible transgenics). Elucidating the etiology of these model systems often includes identification of altered gene expression patterns. The expression of thousands of different genes can be profiled simultaneously using cDNA microarrays (Nat. Genet. 1996 Dec; 14(4): 457). We have identified a set of 8000 mouse ESTs for use in expression pattern profiling and new gene discovery. This set was selected by comparing mouse EST sequences and Genbank entries. 2000 cDNA clones demonstrate sequence identity to mouse mRNA sequences in Genbank (NIH2000 set), 6000 cDNA clones demonstrate sequence similarities to Genbank mRNAs from all species (NIH6000 set). To explore the transcriptional regulation of neural crest development and disease we used high density microarrays on glass slides to perform expression profile analysis. Gene expression was compared between mRNA samples obtained from neural crest derivatives of wild type, Sox10 transcription factor mutant mice (Nat. Genet. 1998 Jan; 18(1): 60) and melanoma susceptible transgenic mice (P.N.A.S. 1996 93: 5866). Neural crest derivatives included melanocyte precursors (melanoblasts), melanocytes and melanoma samples. Comparative analyses identified alterations in known neural crest expressed genes (*Dct, Pmel17, Kit, Met* and *Sox10*), intracellular signaling pathways as well as uncharacterized gene products. We will present hybridization analyses and a hypothesis of how our results fit into a melanocyte/melanoma developmental pathway.

B9. IDENTIFICATION OF PROTEINS THAT INTERACT WITH THE BEIGE (CHEDIAK-HIGASHI SYNDROME) PROTEIN (LYST), USING A YEAST TWO-HYBRID METHOD.

Velizar T. Tchernev, *Krishnan Nandabalan*, Madan Kumar*, Vishnu S. Mishra, Stephen F. Kingsmore*. Departments of Medicine and Pathology, University of Florida; *CuraGen Corporation, New Haven, CT.*

Chediak-Higashi syndrome is an autosomal recessive, immune deficiency disorder of mouse (beige, *bg*) and human (CHS) that is characterized by abnormal intracellular protein transport to and from the lysosome. Recent reports have described the positional cloning of homologous genes, *Lyst* and LYST, that are mutated in *bg* mice and human CHS, respectively. However, since the encoded mouse and human proteins were novel and unlike any of the molecules previously implicated in vesicular transport, their identification did not prove immediately helpful in establishing the precise mechanism whereby beige dysregulates protein transport and lysosomal membrane trafficking. Here we report the identification and confirmation of several known and novel proteins that interact with the Chediak-Higashi protein. Using a modified, improved yeast-two hybrid system, PathCalling, the features of which will be discussed, a human infant brain cDNA library was screened with ~40 baits from the coding domain of LYST and LYST2, a brain specific paralog of LYST. Several proteins, which play important roles in protein transport, such as 14-3-3 and HRS (a component of the t-SNARE protein complex), and in signal transduction, such as calmodulin and casein kinase, a were found to interact with LYST and/or LYST2. Many of these interacting proteins could be linked in a common pathway that acts to regulate of vesicular trafficking and degranulation. Such protein interaction maps, derived by PathCalling, constitute a general method whereby function may be inferred for novel genes.

B. Gene Identification & Mutation Analysis

Abstracts - Posters

B10. ***MSX1*** **IS NOT CRITICAL FOR TOOTH AGENESIS IN STRAIN El MICE.**

Yoshinobu Asada, *Takehiko Shimizu, Kensuke Matsune, Kunihiko Shimizu, Takahide Maeda. Department of Pediatric Dentistry, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba-ken 271, Japan.*

B11. **STUDY ON SPONTANEOUS AND X-RAY INDUCED GERM CELL MUTATIONS IN MICE DETECTED BY COMPUTER ASSISTED TWO-DIMENSIONAL DNA GEL ANALYSIS.**

Jun-ichi Asakawa, *Mieko Kodaira, Hiroaki Katayama, Sachiyo Funamoto, Sachiko Tomita, Dale Preston, Nori Nakamura. Radiation Effects Research Foundation, 5-2 Hijiyama-Park, Hiroshima 732-0815, Japan.*

B12. **CHARACTERISATION OF DEVELOPMENTALLY IMPORTANT GENES MUTATED BY TRANSGENE INSERTION IN A LARGE SERIES OF TRANSGENIC MICE.**

Bennett, W.R., *Ward, A.W. School of Biology and Biochemistry, University of Bath..*

B13. ***or^{2J}*** **MUTANT MICE ARE BLIND AND STERILE DUE TO A LARGE REARRANGEMENT 5' TO THE CHX10 GENE.**

Sharmila Basu[1], Lynda Ploder[2], Muriel Davisson[3], Mark Hankin[4], Rod McInnes[2], **Margit Burmeister[1].** *[1]Mental Health Research Institute, Dept. of Psychiatry, and Dept. of Human Genetics, University of Michigan, Ann Arbor, USA; [2]Dept. of Pediatrics and Genetics, The Hospital for Sick Children, University of Toronto, Canada; [3]The Jackson Laboratory, Bar Harbor, ME, USA; [4]Dept. of Anatomy and Neurobiology, Medical College of Ohio, Toledo, USA.*

B14. **ALTERNATIVE SPLICING FORMS FOR Kif1b ON DISTAL MOUSE CHROMOSOME 4.**

L. Conforti*, *E.A. Buckmaster*, A. Tarlton*, M.C. Brown§, M.F. Lyon#, V.H. Perry*, M.P. Coleman*. Departments of *Pharmacology, §Physiology, University of Oxford, OX1 3QT, UK. #MRC Mammalian Genetics Unit, Chilton, Didcot, Oxon OX11 0RD, UK.*

B15. **dejong**

B16. **TOWARDS POSITIONAL CLONING OF THE MOUSE MUTATION *GUNMETAL*.**

John C. Detter*, *Edward K. Novak**, Vishnu S. Mishra*, Elzbieta B. McMurtrie*, Richard T. Swank**, Stephen F. Kingsmore*. *Department of Medicine and Center for Mammalian Genetics, University of Florida, Gainesville, Florida; **Roswell Park Cancer Institute, Molecular and Cellular Biology Department, Buffalo, New York; CuraGen Corporation, New Haven, CT.*

B17. **POSITIONAL CLONING OF THE BELTED MUTATION: A GENE REQUIRED FOR NORMAL PIGMENT CELL DEVELOPMENT.**

Dorothee Foernzler, *David R. Beier. Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, 20 Shattuck Street, Boston, MA 02115.*

B18. **POSITIONAL CLONING IN GENETICALLY SIMPLE VS COMPLEX EPILEPSIES: A PROSPECTUS.**

Wayne N. Frankel*, *Verity A. Letts, Gregory A. Cox, Marie E. Legare, Connie L. Mahaffey, Frederick S. Bartlett II, Hilary Stanley (epilepsy lab '98). The Jackson Laboratory, Bar Harbor, ME 04609.*

B19. **TISSUE DISTRIBUTION OF Dp260 IN HUMAN AND IDENTIFICATION OF TWO HUMAN Dp260 ISOFORMS.**

R. Gaedigk*, *S.A. Giambrone#, K.M. Fitzgerald*#, R.A. White*. Section of Genetics* and Vision Science Laboratory#, The Children's Mercy Hospital and University of Missouri-Kansas City School of Medicine.*

B20. **TWO NOVEL MOUSE GENES -- Nbps, MAPPED TO THE t-COMPLEX ON CHROMOSOME 17, AND Nbpl, MAPPED TO CHROMOSOME 16 -- ARE DISTINCTIVE NUCLEOTIDE BINDING PROTEINS IN EUKARYOTES.**

Marija J. Grahovac[1], *Hiroshi Nakashima[1], Richard Mazzarella[2], Hiroyuki Fujiwara[1], John R. Kitchen[1], Tracy A. Threat[1], Minoru S. H. Ko[1]. [1]Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48202, USA. [2]Department of Molecular Microbiology, Washington University, St. Louis, MO, USA.*

B21. **MOUSE MUTANTS AFFECTING EARLY EYE DEVELOPMENT.**

J. Graw, *C. Grimm, T. Immervoll, N. Klopp, J. Löster, R. Lutz. GSF-National Research Center for Environment and Health, Institute of Mammalian Genetics, D-85764 Neuherberg, Germany.*

B22. TRANSCRIPTIONAL SEQUENCING: A NEW METHOD FOR DNA SEQUENCING USING RNA POLYMERASE.

Nobuya Sasaki[1,2], Masaki Izawa[1,3], Masanori Watahiki[3], Kaori Ozawa[4], Takumi Tanaka[4], Yuko Yoneda[3], Shuji Matsuura[4], Piero Carninci[1], Masami Muramatsu[1,3], Yasushi Okazaki[1], **Yoshihide Hayashizaki[1,3].** *[1]Genomic Science Center, Genome Science Lab, RIKEN; [2]CREST, JST; [3]Nippon Gene, Co., Ltd., [4]Wako Pure Chemical Industries, Ltd.*

B23. POSITIONAL CLONING OF THE GENE FOR PALLID MUTANT.

Liping Huang, *Jane Gitschier. Howard Hughes Medical Institute; Departments of Medicine and Pediatrics, University of California, San Francisco, CA 94143-0794.*

B24. ABNORMAL TRANSCRIPTS OF MYO5A IN THE NEUROLOGICAL MUTANT FLAILER.

Julie M. Jones[1], *Jing-Dong Huang[2], Nancy Jenkins[2], Neal Copeland[2], Miriam H. Meisler[1]. [1]Department of Human Genetics, University of Michigan, Ann Arbor MI; [2]Mammalian Genetics Division, NCI. Frederick, MD.*

B25. POSITIONAL CLONING OF THE MOUSE JACKSON SHAKER (js) DEAFNESS GENE.

Y. Kikkawa[1], *T. Takada[1], Y. Kohara[1], C. Taya[1], S. Wakana[2], K. Shimizu[3], T. Shiroishi[3], Y. Wakabayashi[4], R. Kominami[4], H. Yonekawa[1]. [1]The Tokyo Met. Inst. Med. Sci., Tokyo 113-0021, Japan; [2]Cent. Inst. Exp. Anim., Kawasaki 216-0001, Japan; [3]Natl. Inst. Genet., Mishima 411-0801, Japan; [4]Niigata Univ. School of Med., Niigata 951-8122, Japan.*

B26. SEQUENCING AND MAPPING OF GENES EXPRESSED IN MOUSE EMBRYOS FROM PREIMPLANTATION STAGES (FROM UNFERTILIZED EGG TO BLASTOCYST).

Minoru S. H. Ko[1], *John R. Kitchen[1], Xiaohong Wang[1], Tracy A. Threat[1], Xueqian Wang[1], Tong Sun[1], Grace E. DePalma[1], Rana Sharara[1], Yuling Liang[1], Jamie Blondin[1], Hirofumi Doi[2]. [1]Doi Bioasymmetry Project, ERATO, JST, Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI 48202, USA, [2]Doi Bioasymmetry Project, ERATO, JST, WBG Marive East 12F, Nakase 2-6, Mihama-ku, Chiba 261-71, Japan.*

B27. CANDIDATES OF THE MOUSE THYMIC LYMPHOMA SUPPRESSOR GENE NEAR THE D12Mit279 REGION.

R. Kominami, *T. Shinbo, A. Matsuki, Y. Matsumoto, S. Kosugi, O. Niwa#. Department of Biochemistry, Niigata University School of Medicine, Asahimachi 1-757, Niigata 951-8122, #Radiation Biology Center, Kyoto University, Yoshida-Konoecho, Sakyou-Ku 606-8315, Japan*

B28. HOTFOOT MOUSE MUTATIONS AFFECT THE δ2 GLUTAMATE RECEPTOR GENE AND ARE ALLELIC TO LURCHER.

Alexis Lalouette, *Jean-Louis Guénet, Sophie Vriz. Unité de Génétique des Mammifères, Institut Pasteur, 25, rue du Dr Roux, 75724 Paris Cedex 15, France.*

B29. MOUSE MODELS FOR VELOCARDIOFACIAL SYNDROME BASED ON COMPARATIVE PHYSICAL MAPS, SEQUENCE ANALYSIS, AND CHROMOSOME ENGINEERING.

J. Lund[1], *B. Roe[2], Z. Wang[2], A. Hua[2], N. Galili[3], S. Baldwin[3], C. Buck[3], A. Wong[3], S. Nayak[3], C. Mickanin[3], M. Budarf[3], B. Emanuel[3], R.H. Reeves[1]. [1]Johns Hopkins U. Schl. Med., [2]U. Oklahoma Adv. Ctr. Genome Tech., [3]Children's Hospital of Philadelphia.*

B30. INITIATION OF THE U.S. MOUSE PHYSICAL MAPPING CONSORTIUM.

John D. McPherson *and the U.S. Mouse Mapping Consortium.*

B31. SKELETAL MUSCLE IN THE MYODYSTROPHY MOUSE SHOWS SARCOLEMMAL DISRUPTION.

K.D. Mathews[1,2], V.W. Straub[3], **K.A. Mills[1],** *K.P. Campbell[3,4]. Depts of Pediatrics[1], Neurology[2], Physiology[3], University of Iowa, Iowa City, Iowa USA, Howard Hughes Medical Institute[4].*

B32. IDENTIFICATION OF MOUSE TRANSCRIBED SEQUENCES BY DIRECT cDNA SELECTION: AN INTERSPERSED REPETITIVE ELEMENT-PCR (IRE-PCR) BASED NOVEL APPROACH.

Vishnu S. Mishra, *John C. Detter, Edward K. Novak* , Sandra M. Holt, Richard T. Swank* Margaret R. Wallace, Frederick S. Southwick, Stephen F. Kingsmore**, Department of Medicine, Center of Mammalian Genetics, University of Florida, Gainesville, *Roswell Park Cancer Institute, Buffalo, New York, **Curagen Corporation, New Haven, CT.*

B33. THE MOUSE Y CHROMOSOME INTERVAL NECESSARY FOR SPERMATOGONIAL PROLIFERATION IS GENE DENSE WITH SYNTENIC HOMOLOGY TO THE HUMAN AZFa REGION.

Sophie Mazeyrat[1], Noëmie Saut[1], Carole A. Sargent[2], Sean Grimmond[3], Guy Longepied[1], Ingrid E. Ehrmann[4], Pamela S. Ellis[4], Andy Greenfield[3], Nabeel A. Affara[2], **Michael J. Mitchell[1].** *[1]INSERM U.491, Faculté de Médecine, 27 Boulevard Jean Moulin, 13385 Marseille cedex 5, France. [2]Human Molecular Genetics Group, University of Cambridge, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP, UK. [3]MRC Mammalian Genetics Unit, Harwell, Didcot, Oxfordshire OX11 ORD, UK. [4]MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK.*

B34. CELL CYCLE REGULATION IN PLASMA CELLS BY ALLELIC VARIANTS OF THE CDKN2A LOCUS, A CANDIDATE GENE FOR PCTR1.

Shuling Zhang, Edward S. Ramsay, **Beverly A. Mock,** *Lab of Genetics, NCI, NIH, Bethesda, MD 20892-4255.*

B35. CDNA NUCLEOTIDE SEQUENCE OF A NEW MOUSE HBB HAPLOTYPE W1 AND ITS EVOLUTIONARY RELATIONSHIP WITH D- AND P- HAPLOTYPE HBB GENES.

Moriwaki, K. (1), *Ueda, Y. (2), Miyashita, N. (3), Shiroishi, T. (4), Yamaguchi, Y. (2). (1) Graduate Univ. Advanced Studies, Hayama, Japan; (2) Fukuyama Univ., Fukuyama; (3) Kagawa Medical College, Kagawa-ken; (4) National Inst. Genet., Mishima.*

B36. ANOMALOUS (PREDUODENAL) PORTAL VEIN; AN AUTOSOMAL RECESSIVE MUTATION IN MICE.

Hiroo Nakajima, *L.Y.Li, Taisei Nomura. Department of Radiation Biology, Faculty of Medicine, Osaka University, B4, 2-2, Yamada-oka, Suita, Osaka 565-0871, JAPAN.*

B37. MOLECULAR CHARACTERIZATION OF *Tlhm1*, A NOVEL GENE EXPRESSED IN MURINE HEMATOPOIETIC TISSUE.

Rhonda H. Nicholson[1], *Serafino Pantano[1], Marija J. Grahovac[1], Tracy A. Threat[1], Joseph Kaplan[2], Minoru S. H. Ko[1]. [1]Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan 48202; [2]Children's Hospital of Michigan, Detroit, Michigan 48210.*

B38. MOLECULAR CHARACTERIZATION OF DELETIONS ON MOUSE CHROMOSOME 11 CONTRIBUTING TO α-THALLASEMIA AND PERIMPLANTATION LETHALITY.

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B39. THE MOUSE MUTANT *mceph*, WITH ENLARGED BRAIN, HAS DISTURBANCES IN THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM.

Susanna Petersson, *Martin Schalling, Catharina Lavebratt. Neurogenetics Unit, Center for Molecular Medicine, Karolinska Hospital L8:00, S-171 76 Stockholm, Sweden.*

B40. GLUTATHIONE REDUCTASE ACTIVITY DEFICIENCY IN *Mus musculus*: A MODEL FOR THE HUMAN DISEASE.

Walter Pretsch. *GSF - National Research Center for Environment and Health, Institute of Mammalian Genetics, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany.*

B41. MUTATIONS IN THE UNCONVENTIONAL MYOSIN *Myo15* CAUSE DEAFNESS IN *shaker-2* and *shaker-2^J* MICE, MODELS FOR THE HUMAN NONSYNDROMIC DEAFNESS DFNB3.

Frank J. Probst, *Robert A. Fridell, Yehoash Raphael, Aihui Wang, Yong Liang, Konrad Noben-Trauth, David F. Dolan, Thomas B. Friedman, Sally A. Camper.*

B42. *PAX6^{4NEU}*: A HYPOMORPH ALLELE IN THE MOUSE DUE TO AN AMINO ACID SUBSTITUTION IN THE HOMEOBOX DOMAIN.

R. Sandulache, *A. Neuhäuser-Klaus, T. Hermann*, J. Favor. GSF-Institute of Mammalian Genetics, Neuherberg, Germany; *Institute of Molecular and Cellular Biology, Strasbourg, France.*

B43. THREE GENE NEIGHBORS IN 40 kb OF THE MOUSE t-COMPLEX -- TWO NEW GENES (Nbps and D17Wsu15e) AND ONE KNOWN (Als) -- SHOW HEMIZYGOUS DNA METHYLATION PATTERNS.

Tokihiko Shimada[1], *Hiroshi Nakashima*[1], *Rhonda H. Nicholson*[1], *Marija J. Grahovac*[1], *Ramaiah Nagaraja*[2], *Ellson Chen*[3], *Chun-Nan Chen*[3], *David Schlessinger*[2], *Minoru S. H. Ko*[1,2]. [1]*Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan 48202, USA.* [2]*NIH/National Institute on Aging, Baltimore, MD 21224, 3PE-Applied Biosystems, Foster City, CA 94404*

B44. LINKAGE ANALYSIS OF THE GUTTER SHAPED ROOT (*GSR*) ON MOUSE CHROMOSOME 5.

Takehiko Shimizu, *Kensuke Matsune, Kunihiko Shimizu, Yoshinobu Asada, Takahide Maeda. Department of Pediatric Dentistry, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba-ken 271-8587, Japan.*

B45. CHARACTERISATION OF GENES ISOLATED FROM THE BROWN DELETION COMPLEX.

Eleanor H. Simpson[1], *Dabney K. Johnson*[2], *Pat Hunsicker*[2], *Ruth Suffolk*[1], *Emily D. Griffith*[2], *Ian Jackson*[1]. [1]*MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU.* [2]*Biology Division Oak Ridge National Laboratory, Oak Ridge, Tennessee, US.*

B46. THE TIGHT SKIN PHENOTYPE OF Tsk/+ MICE IS NOT DEPENDENT ON THE PRESENCE OF MATURE T AND B LYMPHOCYTES.

Linda D. Siracusa[1], *Rodney McGrath*[2], *Jill K. Fisher*[1], *Michael Raghunath*[3], *Cay M. Kielty*[4], *Sergio A. Jimenez*[2]. [1]*Department of Microbiology and Immunology, Kimmel Cancer Center;* [2]*Department of Rheumatology, Jefferson Medical College, 233 South 10th Street, Philadelphia, PA 19107 USA;* [3]*Department of Dermatology, University of Muenster, D-48149, Germany;* [4]*School of Biological Sciences, University of Manchester, Manchester, M13 9PT United Kingdom.*

B47. MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AN IMPRINTED REGION OF MOUSE CHROMOSOME 7 AND RELATED SEGMENTS OF HUMAN CHROMOSOME 19.

Joomyeong Kim, Xiaochen Liu, **Lisa Stubbs.** *Human Genome Center, L-452, Lawrence Livermore National Laboratory, Livermore, California 94551.*

B48. IDENTIFICATION OF A DELETION AND A TANDEM DUPLICATION IN ALLELES OF THE PEARL (*Ap3bl*) GENE.

Lijun Feng[1], *Albert B. Seymour*[3], *Agnes To*[2], *Edward K. Novak*[1], *Andrew A. Peden*[5], *Nicholas A. Bright*[5], *Margaret Robinson*[5], *Michael Gorin*[2], *Eva M. Eicher*[4], *Rosemary W. Elliott*[1], **Richard T. Swank**[1]. [1]*Dept. of Molecular Cell Biology, Roswell Park Cancer Institute,* [2]*Dept. of Ophthalmalogy and Human Genetics, Univ. of Pittsburgh,* [3]*Dept. of Molecular Sciences, Pfizer Central Research,* [4]*The Jackson Laboratory,* [5]*Dept. of Clinical Biochem., Univ. of Cambridge.*

B49. IDENTIFYING INTERACTING PARTNERS TO THE MOUSE MYOSIN VIIA PROTEIN INVOLVED IN GENETIC DEAFNESS.

Penio Todorov[1], *Xue Liu*[1], *Dennis Saw*[1], *Karen Steel*[2], *Steve Brown*[1]. *1. MRC Mouse Genome Centre and Mammalian Genetics Unit, Harwell, OX11 ORD, UK. 2. MRC Institute of Hearing Research, University Park, Nottingham, NG7 2RD, UK.*

B50. POSITIONAL CLONING AND MOLECULAR ANALYSIS OF THE MOUSE DEAFNESS MUTATION (*wi*).

A. Varela[1], *P. Mburu*[1], *H.T. Tsai*[1], *A.J.W. Paige*[1], *S.D.M. Brown*[1], *B.W. Kiernan*[2], *J. Fleming*[2], *M. Rogers*[2], *D. Hughes*[2], *R. Hardisty*[2], *K.P. Steel*[2]. [1]*MRC Mouse Genome Centre and Mammalian Genetics Unit, Harwell, Oxon., OX11 0RD, UK.* [2]*MRC Institute of Hearing Research, Nottingham, NG7 2RD, UK.*

B51. A NOVEL TYPE OF MYOSIN, MYOSINXV ENCODED BY THE MOUSE DEAFNESS GENE shaker-2.

Y. Wakabayashi[1], *Y. Takahashi*[1], *Y. Kikkawa*[2], *H. Okano*[2], *Y. Mishima*[1], *H. Yonekawa*[2], *R. Kominami*[1]. [1]*Department of Biochemistry, Niigata University School of Medicine, Asahimachi 1-757, Niigata 951-8122, Japan.* [2]*Tokyo Metropolitan Institute, Bunkyoku, Japan.*

B52. EVOLUTION OF A YOUNG MURINE GENE FAMILY BY GENE FUSION AND AMPLIFICATION.

Dieter Weichenhan, *Bärbel Kunze, Heinz Winking, Walther Traut. Medizinische Universität zu Lübeck, Institut für Biologie, Ratzeburger Allee 160, D-23538 Lübeck, Germany. email: weichenh@molbio.mu-luebeck.de*

B53. ANTISENSE TRANSCRIPTS OF *Gnas*, AN IMPRINTED GENE ON MOUSE CHROMOSOME 2.

Christine A. Wells, *Simon T. Ball, Stephanie F. Wroe, Christine M. Williamson, Terry Hacker, Ann-Marie Mallon, Richard Trembath*, Josephine Peters. Mammalian Genetics Unit, MRC, Harwell, Didcot, Oxfordshire, OX11 ORD, UK. *Department of Genetics and Medicine, University of Leicester, Leicester, LE1 7RH, UK.*

B54. IDENTIFICATION OF IMPRINTED GENES ON DISTAL MOUSE CHROMOSOME 2 BY SUPPRESSION SUBTRACTIVE HYBRIDISATION AND A CANDIDATE GENE APPROACH.

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B10. *MSX1* IS NOT CRITICAL FOR TOOTH AGENESIS IN STRAIN El MICE.

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Others than for man and mouse, descriptions of tooth absence in mammalian dentitions are rare. We have been studied the morphological features of the molar teeth in the upper and lower jaw of several inbred mice. We noted about 90-100% absence of the third molars on both the maxilla and mandible of strain El/sea mice. We reported that the El strain was one of the most useful models for studying the tooth germ development at an early age. In recent studies, a transcription factor, *MSX1* has been shown to have function of direct epithelial-mesenchymal interactions that initiate tooth formation in humans and mice. It has been reported that an Arg→Pro substitution within the homeodomain of human *MSX1* causes selective tooth agenesis for second premolar and third molar. *MSX1*-deficient mice exhibit multiple craniofacial abnormalities including complete tooth agenesis. The purpose of this study is to confirm whether El mice have the Arg→Pro substitution within the homeodomain of murine *MSX1*. To identify the murine *MSX1* gene structure in El mice that have tooth agenesis, we performed the PCR analyses using three sets of specific primers. Primers (F, forward; R, reverse) used were; exon1: 1F (ATGACTTCTTTGCCACTC),1R(GTCCGATCTAGTTTCTCG);exon2:2F(CTACGCAAGCACAAGACCA),2R(CTGC TCTGCTCAAAGAGGTG); Homeodomain of exon2: 2HF (GACGCCTTTCACCACAG), 2HR (TGCAGTCTCTTG-GCCTT). These primers were designed with Macintosh software, 4.0. It was observed that there were no different gene structures of *MSX1* in El mice as compared with those in other inbred strains of mice with normal development for teeth formations. No cut site of *Ban*II restriction enzyme in homeodomain within exon2 of *MSX1* was presented in the DNAs obtained from El mice and controls. Therefore, we investigated to confirm that El mice did not have the homeodomain mutation on *MSX1*, which cause tooth agenesis in humans. The exon2 PCR products were sequenced directly with dye terminator labelling in an ABI 310 genetic analyzer (Perkin Elmer). It was observed that the G→C transversion in homeodomain within exon2 of *MSX1* did not imply in El mice. Based on these observations, we propose that *MSX1* is not critical for tooth agenesis in the third molar of strains El mice.

B11. STUDY ON SPONTANEOUS AND X-RAY INDUCED GERM CELL MUTATIONS IN MICE DETECTED BY COMPUTER ASSISTED TWO-DIMENSIONAL DNA GEL ANALYSIS.

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We have successfully detected spontaneous and X-ray induced germ cell mutations in mice by a computer assisted two-dimensional DNA gel analysis. Also we have successfully isolated and characterized the normal and/or mutated DNA fragments and demonstrated the power as well as usefulness of our approach in mutation screening.

A principal focus of our research group is the study of human germinal mutations induced by ionizing radiation from Atomic-bombs. Recently we have developed a vertical two-dimensional electrophoresis (2DE) system of end-labeled *Not*I fragments at RERF. The most distinctive advantage of this method is that over 1000 DNA fragments (spots) are provided on a single gel without using any probes. The specific labeling of the *Not*I ends also permits the quantitative estimation of copy number of each DNA fragment from each spot intensity. We have implemented a computer based approach for the quantitative analysis of the 2DE patterns to assess the detectability of germ cell mutations in mice. We examined F1 mice (BALB/c) from spermatogonial cells irradiated with 5 Gy of X rays and control F1s born to the same males but before irradiation. We prepared two kinds of 2DE gels for each DNA sample, one for 1-5 kb and the other for 5-9 kb *Not*I/*Eco*RV DNA fragments. Autoradiograms were digitized with a scanner and approximately 1100 DNA spots were visualized on each 2DE preparation. To detect mutations associated with a 50% decrease or increase in the spot intensity, the coefficient of variation of spot intensity should be less than 0.12, and 510 and 580 spots, respectively, meet this criteria. By now, we have analyzed 92 gels and surveyed 50,018 spots from the control and 74,028 spots from 132 gels of the exposed group. In the control group, five mutant spots, each with half spot intensity of the normal resulting from a mutation, were detected in three F1s. Among these, four mutant spots were detected in two F1s derived from the same father. The two F1s had the same mutations in the same two spots. These results imply that these four mutations are clusters and are derived from a single mutational event. In the exposed group, eight mutant spots were detected in eight F1s. Among these, two occurred in the same spot in two F1s derived from the same father and three occurred in the same spots in three F1s in another but in the same Family. Thus, we observed independent five mutational events involving two cluster mutations in the 5-Gy exposed group.

We have cloned the normal and/or the mutated DNA fragments from the DNAs extracted from the 2D gel spots. Molecular analysis revealed that one mutation in the exposed group was insertion-type mutation and the remaining four were deletion-type mutations. The two mutations in the control were both deletion-type mutations.

B12. CHARACTERISATION OF DEVELOPMENTALLY IMPORTANT GENES MUTATED BY TRANSGENE INSERTION IN A LARGE SERIES OF TRANSGENIC MICE.

Bennett, W.R., *Ward, A.W. School of Biology and Biochemistry, University of Bath..*

67 transgenic mouse lines have been screened for transgene-associated insertional mutations. Partial screening for pre- and peri-natal phenotypes has been performed during re-derivation of the lines by Caesarean section, but several post-natal mutant phenotypes have been isolated by breeding to homozygosity. Two of these mutant phenotypes have now been characterised and tentatively mapped by FISH. The Harry line of transgenic mice has a transgene integration site on Chr 15 which causes a recessive piebald lethal phenotype. This is probably due to some disruption at or near the *Sox10* locus. The Holly line of transgenic mice has a transgene integration site on Chr 1 which gives rise to a recessive phenotype resembling mutants at the *Cat-4* locus on Chr 8, with iris dysplasia, corneal opacity visible from around 15 days post-partum and a progressive anterior sub-capsular cataract. This may be allelic with *Cat-2* on Chr 1.

B13. *or^{2J}* MUTANT MICE ARE BLIND AND STERILE DUE TO A LARGE REARRANGEMENT 5' TO THE CHX10 GENE.

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Ocular retardation is a murine mutation characterized by micropthalmia, a thin, poorly differentiated retina, and blindness. Earlier studies by our group have shown that a mutation in the *Chx10* homeoprotein-encoding retinal gene is responsible for the phenotype of mice homozygous for the orJ allele of ocular retardation. Upon sequencing of all exons and exon-intron boundaries from the genomic DNA of *or^{2J}* mice, however, no mutations were found. In addition to micropthalmia and retinal defects, *or^{2J}* homozygotes, in contrast to *or^{J}* mice, are also sterile (both sexes). We hypothesized that the *or^{2J}* mutation involved either an intronic insertion or deletion, or a larger-scale genomic rearrangement at the molecular level, perhaps involving a second gene. Molecular analysis of the *Chx10* genomic region of *or^{2J}* identified a large rearrangement, detectable by pulsed field gel electrophoresis, involving a region significantly 5' to the *Chx10* gene. In addition, we have conducted RNA and protein analysis to determine whether any Chx10 transcript is present in *or^{2J}* homozygotes. We have analyzed the sterility phenotype genetically and histologically, to determine conclusively whether this phenotype cosegregates with the eye phenotype. We postulate that the large rearrangement involves a second gene causing the sterility of *or^{2J}* mice.

B14. ALTERNATIVE SPLICING FORMS FOR Kif1b ON DISTAL MOUSE CHROMOSOME 4.

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Kinesin proteins are microtubule-associated molecular motors that play an important role in intracellular transport and cell division. They are microtubule-activated ATPases composed of a motor domain, that binds to microtubules, and a cargo-binding domain, that binds to specific organelles. After the identification of kinesin, many more members of the superfamily have been described and their specificity for different cargoes has been shown (Moore and Endow, 1996; Hirokawa, 1996, 1998).

While searching for candidate genes for slow Wallerian degeneration on distal mouse Chromosome 4 (Lyon et al., 1993), we have isolated a series of cDNA clones covering more than 8 kb with an open reading frame of 5474 bp. The gene product is a member of the kinesin superfamily with a motor domain almost identical to Kif1b (Nangaku et al, 1994), but with a novel C-terminal region, which is homologous to Kif1a. This Kif1b sequence detected only single copy fragments in genomic DNA, indicating that there is only one *Kif1b* gene. Only the novel 3'-end could be found in cDNA library screening and northern blots from mouse brain, although the reported 3'end was also detected by RT-PCR. Therefore, we believe that the novel C-terminal region is incorporated in an alternatively spliced form of Kif1b that accounts for the most part of Kif1b's expression. In addition, we found more alternatively spliced exons that can give rise to heterogeneous transcripts. Since in this type of kinesin the C-terminal domain is the cargo-binding domain, some of the alternative forms might be used to carry different cargoes.

The map location of this gene agrees with the previous report of *Kif1b* on mouse chromosome 4 (Nagakawa et al., 1997), but we have also refined the map position to the *Wld* genetic candidate interval; it lies outside the recently identified triplication (Coleman et al., 1998). The locus order on this interval is Centromere-Nppa-*D4Mit225-Wlds* triplication- *Kif1b- Pgd-D4Mit310*-Telomere. The gene order NPPA-KIF1B-PGD is likely to be conserved in human Chromosome 1p36, a region of conserved synteny with mouse distal Chromosome 4.

We have sequenced this new form of Kif in the wild type and Wlds mouse, but no difference in sequence could be found; moreover, northern blot analysis showed that there is no difference in the level of expression or in the size of the transcript between the two mice, indicating that *Kif1b* is unlikely to be the *Wld* gene. However, an axonal form of the inherited peripheral neurodegenerative disease Charcot-Marie-Tooth (CMT2A) has been mapped to the homologous location in man, so the human homologue, KIF1B, remains a good candidate for this disease.

Acknowledgements: This work was supported by Bristol-Myers Squibb and the Blaschko Visiting Research Scholarship.

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B16. TOWARDS POSITIONAL CLONING OF THE MOUSE MUTATION *GUNMETAL*.

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Gunmetal (*gm*) is an autosomal recessive mouse mutation on central mouse chromosome (Chr) 14. *gm* homozygotes exhibit partial albinism, platelet defects, high mortality, and poor fecundity. Characteristics of *gm* platelets include: 1) macrothrombocytopenia; 2) deficiency of both dense and (-granule constituents; and 3) abnormal expression of platelet low molecular weight GTP-binding proteins. These defects are associated with prolonged bleeding time, and suggest *gm* as a possible animal model for gray platelet syndrome (GPS), a platelet (-granule storage pool deficiency (α-SPD) in humans.

In order to identify the gm locus, we have taken a positional cloning approach. Previous mapping efforts have localized gm to a 1.5 cM interval on central mouse Chr 14, between *Myhca* and *D14Mit122*, in 727 backcross [(C57BL/6J-*gm* x PWK)F1 X C57BL/6J-*gm*] animals. Recently, we have narrowed this to a 0.6 cM interval, between *D14Sfk8* and *D14Sfk9*, in an additional 500 backcross [(C57BL/6J-gm x DBA)F1 X C57BL/6J-*gm*] animals. Using these flanking genetic markers, as well as other sequence-tagged sites (STS), a well-defined yeast artificial chromosome (YAC) contig spanning the ~500 kb *gm* critical region has been constructed.

To generate novel, PCR-amplifiable, simple sequence length polymorphisms (SSLPs) that narrow the *gm* critical region further, we performed interspersed repetitive element (IRE)-PCR with out-looking, mouse B1 repetitive element primers using gm critical region YAC DNA as template. This method allowed identification of four novel SSLPs, *D14Sfk1, 2, 3, & 5*, that lie within the *gm* critical region. It is hopeful that these new genetic markers will aid in narrowing the *gm* critical region.

We have also utilized a novel direct cDNA selection method to identify cDNA fragments adjacent to repetitive elements within YACs from the *gm* critical region. Using this approach, we have isolated six cDNA fragments that lie within the *gm* critical region. Sequencing revealed that two of these fragments are from known genes and the other four are novel. Further analysis of these candidates is underway. Identification of the gene mutated in gm mice will enable better understanding of the regulation of platelet production and may offer new insights into treatment of platelet storage pool deficiency.

B17. POSITIONAL CLONING OF THE BELTED MUTATION: A GENE REQUIRED FOR NORMAL PIGMENT CELL DEVELOPMENT.

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The recessive murine coat color mutation *belted* exhibits a specific defect in the development of neural-crest-derived pigment cells. *bt* mice form a white patch of hair transversely across the back posterior to the midline that is often joined around the body to form a belt. This phenotype results from the absence of melanocytes in specific body areas, presumably due to a failure to migrate or proliferate early in development. A similar phenotype is observed in the white-spotting mutant W^{sash}. In this mutation the receptor tyrosine kinase *Kit* is expressed abnormally; the similarity in phenotype suggests that the *bt* product may be involved in the *Mgf/Kit* signalling pathway.

The original spontaneous mutation at bt has been mapped to chromosome 15. We discovered a new allele of *bt* as one of an unexpectedly large number of new mutations thathave occurred in derivatives of a transgenic mouse line, but are independent from the transgene insertion. One attractive hypothesis to explain the apparent elevated mutation frequency in this line is that the transgene insertion is associated with activation of a transposable genetic element, which is integrating elsewhere in the genome causing mutations.

The positional cloning of the *bt* gene will allow us to study its important role in pigment cell development, and to explore the possibility that our bt mutation is generated by an insertion event. High resolution mapping using PCR-based microsatellite linkage analysis localized the *bt*-containing region to a 0.5 cM interval on distal mouse chromosome 15. A 280kb YAC clone was isolated that carried the two *bt*-flanking markers, and a BAC-contig was established covering this region. Sample sequencing of 3 BAC clones has identified candidate EST clones, which are currently being characterized in several *bt* mutants. As an additional approach to identify candidate genes, cDNA selection using the 3 BAC clones of the *bt*-containing region is currently in progress.

B18. POSITIONAL CLONING IN GENETICALLY SIMPLE VS COMPLEX EPILEPSIES: A PROSPECTUS.

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This presentation will compare and contrast our lab's efforts towards identifying genes that underly mouse models for epilepsy. For this, we have undertaken two different approaches. In the first, (QTL mapping - congenic strains - congenic recombinant fine-mapping), we have focused on a major effect QTL called El2 (Chr 2) defined as a natural variant between inbred mouse strains EL and ABP. In the second, (spontaneous single gene mutations), most of our efforts has been on slow-wave epilepsy and stargazer. Although the simple traits have met with success (1,2), the complex have proven to quite a challenge mainly because multiple genes underly "single" QTL and some interact with strain background in ways that could not be anticipated from the initial crosses. The differences in the two approaches and varying successes will be compared and contrasted. In addition, in view of the number of genes which, when mutated, are likely to influence seizure susceptibility, the prospects of applying newer strategies (e.g. mutagenesis) towards identifying these genes will be presented.

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[Supported by NIH grants NS31348, NS32801 and a Klingenstein Fellowship to WNF.]

B19. TISSUE DISTRIBUTION OF Dp260 IN HUMAN AND IDENTIFICATION OF TWO HUMAN Dp260 ISOFORMS.

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Dystrophin (427 kDa) is a spectrin-like cytoskeleton protein which provides structural support for muscle cells as exemplified by its involvement in Duchenne muscular dystrophy (DMD). A novel human dystrophin isoform called dystrophin protein-260 (Dp260; 260kDa) has been identified, cloned, and characterized by our laboratory. Dp260 possesses a hydrophilic N-terminus consisting of 13 amino acids which is derived from the splicing of a unique first exon (called R1B) to exon 30. Sequence analysis of a cDNA clone as well as a genomic clone identifies the deduced amino acid sequence of the Dp260 specific portion of the molecule in human to be identical to the mouse sequence. RT-PCR was performed on samples of both species with total RNA from brain, heart, liver, lung, spleen and testis using specific primers in the 5' UTR of Dp260 R1 and exon 31. Dp260 was identified in all but the liver samples in human and mouse after Southern blotting the PCR products and hybridization with [^{32}P] labeled oligonucleotides specific for the Dp260 R1 region.

Screening of a human retinal cDNA library revealed a second novel isoform with a predicted molecular weight of 271kDa. This isoform would have a 16 amino acid hydrophobic N-terminus (called R1A). The Dp260-1 isoform is derived from utilizing only the R1A (95bp) portion of the R1 exon whereas the Dp260-2 isoform is created by using the full R1 exon (R1A portion and the remaining sequence in the R1B segment).

From Draft

Splicing model of exon R1 to exon 30 and structure of the 5' end of the Dp260 transcription unit. The first exon is alternatively spliced by the use of two donor sites joined to a common acceptor site on exon 30. Use of the A segment of exon R1 eliminates the 143bp B (stippled) portion. The 95bp alternatively spliced portion of exon R1 A includes a potential start codon at position 48. Alternatively, the full 238bp R1 exon includes a second ATG at position 200 in frame with the downstream exon 30.

B20. TWO NOVEL MOUSE GENES -- Nbps, MAPPED TO THE t-COMPLEX ON CHROMOSOME 17, AND Nbpl, MAPPED TO CHROMOSOME 16 -- ARE DISTINCTIVE NUCLEOTIDE BINDING PROTEINS IN EUKARYOTES.

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Sequencing of a mouse ectoplacental cone (EPC) cDNA library, which represents genes expressed at the maternal-fetal interface during the peri-implantation period, generated 3186 ESTs, from which 155 new genes were mapped on the Jackson Laboratory Interspecific Backcross Mouse Panels. Ten novel genes were found to map clustered at the t-complex of Chromosome 17, and one of the ten showed strong sequence similarity to a predicted yeast gene, *YIA3w*. *YIA3w* belongs to the *NBP/MRP* family, which includes human *NBP*, yeast *NBP35*, and prokaryote *MRPs*. The *NBP/MRP* family is characterized by motifs common to bacterial ATPases, as well as other common amino acid sequences of unknown function. The gene was named *Nbps* (for nucleotide binding protein short form), because the predicted protein sequence lacked an additional amino acid sequence found in human *NBP*. Yeast *YIA3w* is similarly shorter than the yeast *NBP35*. The additional sequence, in the N-terminal region, includes 4 cysteine residues. A search in dbEST identified the mouse homologue of human *NBP* and yeast *NBP35*, and it was named *Nbpl* (nucleotide binding protein long form). Both *Nbps* and *Nbpl* are ubiquitously expressed in adult tissues and in staged embryos. *Nbps* has been mapped to Inversion 3 of the t-complex or an adjacent region, close to known imprinted genes, *Igf2r* and *Mas1* (see Shimada et al., these Abstracts, for methylation studies of *Nbps*). *Nbpl* has been mapped to the proximal region of Chromosome 16; its methylation status is unknown.

We have isolated the human ortholog of mouse *Nbps* and named it human *NBPS*. Expression of *hNBPS* is ubiquitous in a wide range of adult and fetal tissues. We have also sequenced *hNBPS* and predicted its protein sequence. Homology searches have allowed us to distinguish the long and short forms of Nbp as two distinct groups within the *Nbp* family. Based on the construction of an evolutionary phylogenetic tree, the existence of one form of *MRP* (*NBP*) is obvious in prokaryotes, whereas two forms emerge in eukaryotes. The eukaryotic short form of *NBP* includes: *hNBPS*, *mNbps*, *YIA3w*. The eukaryotic long form includes: *hNBP*, *mNbpl*, *NBP35*, *F1068.6* (*C. elegans*). At least one form of *MRP* has been found in the following prokaryotic species: *M. jannaschii*, *H. influenzae*, *E. coli*, *B. subtilis*, *P. fragi*, *M. leprae*, and *Synechocystis*. Our findings suggest that every eukaryote has both a long and short form of the nucleotide binding protein.

The role of the *NBP* or *MRP* family is yet to be elucidated. Sequence analysis has shown that some of the N-terminal amino acid residues, including the 4 cysteines, are widely conserved evolutionarily, as are an ATP-GTP binding site and an *MRP* motif (PROSITE). Based on their conservation and ubiquity, *Nbps* and *Nbpl* may be involved in a fundamental cellular function, possibly in the cell cycle.

B21. MOUSE MUTANTS AFFECTING EARLY EYE DEVELOPMENT.

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The lens plays an essential role for proper eye development. Mouse mutants effecting lens development are excellent models for corresponding human disorders. The mutant aphakia (*ak*) was characterized as lens-less, and the corresponding gene was mapped to chromosome 19 (1). Recent investigations in our laboratory demonstrated *ak* between the microsatellite markers *D19Mit10* and *D19Mit4/D19Mit91*. *Fgf8* and *Chuk1* have been excluded as candidate genes. Lens development is arrested during formation of the lens vesicle at the lens stalk stage accompanied by an altered *Pax6* expression pattern. The lens defect leads to irregular iris development and retinal folding. At the corresponding human chromosome 10q24 only the corneal dystrophy of the Thiel-Behnke type exhibits an ocular phenotype. Congenital aphakia is known as a rare human anomaly.

Two mutant alleles of the *Cat3* locus have been mapped to mouse chromosome 10 in a distance of less than 0.3 cM to the microsatellite markers *D10Mit41* and *D10Mit95*. *Ldc, Dcn, Tr2-11* and *Elk-3* have been excluded as candidate genes. (2). An additional cell layer between the anterior lens epithelium and the primary lens fibers leads to vacuolization of the lens and s.econdary malformations of the cornea and iris. Since *Cat3* is mapped to a position corresponding to human chromosome 12q21-24, the disorder cornea plana congenita can be discussed as a candidate disease.

The series of *Cat2* mutations have been mapped to the locus encoding the γ-crystallin gene cluster *Cryg* at mouse chromosome 1 (3). The *Cat2^{nop}* mutation is characterized by a deletion of 11 bp and an insertion of 4 bp in the 3rd exon of *Crygb* leading to a truncated γB-crystallin. The defect in the *Crygb* gene is causative for the stop of the secondary lens fiber cell differentiation without effects on other ocular tissues. Further *Cat2* mutations have been characterized in the *Cryga* and *Cryge* genes. These mouse mutants are interesting models for corresponding human cataracts caused by mutations in the *CRYG* genes at human chromosome 2q32-35.

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B22. TRANSCRIPTIONAL SEQUENCING: A NEW METHOD FOR DNA SEQUENCING USING RNA POLYMERASE.

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We have developed a novel sequencing method based on the RNA polymerase (RNAP) chain termination reaction with rhodamine dye attached to 3′ deoxynucleoside triphosphate (dye-3′ dNTP). this method enables us to conduct a rapid isothermal sequencing reaction in less than 30 minutes, to reduce the amount of template required, and to do PCR direct sequencing without the elimination of primers and 2′ dNTP which disturbs the Sanger sequencing reaction. An accurate and longer read length was made possible by newly designed four-color dye-3′ dNTPs and mutated RNAP with an improved incorporation rate of 3′ dNTP. This method should be useful for large-scale sequencing in genome projects and clinical diagnosis.

B23. POSITIONAL CLONING OF THE GENE FOR PALLID MUTANT.

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The pallid mutant, which was found in the wild, is one of the platelet storage pool deficient (SPD) mouse mutants. It is an autosomal recessive mutant characterized by prolonged bleeding time, pigment dilution, kidney lysosomal enzyme elevation, α-1-antitrypsin deficiency, and abnormal otolith formation. The pallid mutation was made congenic with C57BL/6J by backcrossing into that strain for 45 generations and was mapped on chromosome 2 at 68 cM. A YAC contig which covers the pallid region, as determined by ancestral chromosome mapping, was generated. The region extends ~1 Mb from *Epb4.2* to *β2M*. A mouse liver cDNA library was screened with a YAC DNA from this contig and six independent genes (one of later proved to be the lethal milk gene) were discovered. The cDNAs derived from C57BL/6J and pallid mutant which cover the open reading frame of the individual genes were amplified and sequences were subsequently compared. One of the cDNA derived from the pallid mutant was found to have a C to T transition at an arginine codon yielding a stop codon. The cDNA is ~ 4 kb in size and the derived amino acid sequence predicts a short, highly charged protein with no homology to any known proteins in the databases. The change also causes skipping of the nonsense codon-containing exon in ~20% of the transcripts in pallid mutant, which also leads to a premature translational termination. Because of the similarity in phenotype of pallid to mocha and pearl, we hypothesize that the pallid protein may play a role in protein trafficking, potentially interacting with the clatherin/AP-3 complex.

B24. ABNORMAL TRANSCRIPTS OF MYO5A IN THE NEUROLOGICAL MUTANT FLAILER.

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The recessive mutant flailer (gene symbol *flail*) was formerly designated tumbler 2J. We reported last year that flailer is closely linked to the dilute (*Myo5a*) locus on mouse chromosome 9. flailer homozygotes exhibit transient ataxia around the time of weaning that closely resembles the phenotype of the neurological alleles of dilute. There is no visible alteration of pigmentation in flailer homozygotes, unlike the other characterized alleles of dilute. No coding mutations were identified when the entire coding region of the *Myo5a* transcript was amplified from homozygous mutant brain and sequenced. When Northern blots of polyA RNA were probed with a partial *Myo5a* cDNA containing the head region of the coding sequence, the hybridizing fragments in mutant and normal brain were identical. However, hybridization of the same blots with cDNA probes containing the tail region of *Myo5A* detected three additional transcripts in the mutant. Experiments are in progress to determine the molecular origin of the abnormal transcripts. The data suggest that production of an abnormal protein is responsible for the phenotype of flailer mice, in the presence of the wildtype protein.

B25. POSITIONAL CLONING OF THE MOUSE JACKSON SHAKER (js) DEAFNESS GENE.

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The Jackson shaker (*js*) is a recessive mutation which is located in the distal part of the mouse chromosome 11. The homozygotes exhibit hyperactivity, head-tossing and circling behavior due to vestibular dysfunction, together with cochlea defects involving degeneration of stereocilia of the outer hair cells and dysfunction of organ of Corti. A physical map with 1.3-mb distance was constructed on the *js* region. Twelve expressed transcripts were identified from the region by exon trapping and cDNA selection. One out of the twelve, showed a T to C transition specific for the *js* mutation by PT-PCR sequencing. A probe obtained from this product allowed us to isolate a 2.2-kb cDNA as a strong candidate from a mouse inner ear cDNA library kindly given by Dr. S. Brown. The *js* candidate encodes a microtubles-oriented motor protein. This is the first finding that such motor protein is involved in the auditory transduction.

B26. SEQUENCING AND MAPPING OF GENES EXPRESSED IN MOUSE EMBRYOS FROM PREIMPLANTATION STAGES (FROM UNFERTILIZED EGG TO BLASTOCYST).

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We have been extensively sequencing and mapping cDNA cohorts derived from preimplantation mouse embryos. Seven cDNA libraries were constructed with an average insert size of 1.5 kb and arrayed in 96-well microtiter plates. Single pass sequencing from the 3'-ends have yielded 3'-expressed sequence tags (ESTs), which are more suited to identify and map genes than are 5'-ESTs. So far we have collected 22,527 ESTs with excellent sequence quality (<3% Ns) over tracts on the order of 500 nt. All of these ESTs have been deposited in the public EST database and are available through NCBI's Entrez or Blast search systems (dbEST:[http://www.ncbi.nlm.nih.gov/]). The numbers of 3'-ESTs and fraction of "unknown" genes from the individual cDNA libraries are as follows: (1) unfertilized eggs (3,096 ESTs; 74%), (2) fertilized eggs (3,314 ESTs; 2,041; 62%), (3) 2-cell embryos (3,684 ESTs; 57%) (4) 4-cell embryos (Processing), (5) 8-cell embryos (3,444 ESTs; 60%), (6) Morula (16-cell) embryos (3,297 ESTs; Processing), (7) 3.5-dpc blastocysts (16 - 32 cells) (5,692 ESTs; 56%). These data indicate that cDNA libraries from the preimplantation mouse embryos are an excellent source for finding large numbers of expressed genes that have not been recovered in other cDNA collections. We are currently developing expression profiles for the ESTs throughout preimplantation development.

By removing redundant representation from the collection of 22,527 ESTs, we have inferred a set of about 5,000 unique and "unknown" ESTs. Those ESTs were used to design and synthesize PCR primer pairs. About 700 ESTs were mapped on the Jackson Laboratory BSS Interspecific Backcross Mouse Panels, using a heteroduplex method to discriminate between the C57BL/6J and M. spretus alleles. The map information is accessible through a WWW server [http://www.jax.org/resources/documents/cmdata/BSS.html]. In addition, the ESTs for all these primer pairs are being mapped on the Radiation Hybrid Panels by the MRC UK Mouse Genome Center. The ESTs will then provide candidates for positional cloning projects as well as useful anchors relating the genetic, RH, and physical YAC/BAC maps.

B27. CANDIDATES OF THE MOUSE THYMIC LYMPHOMA SUPPRESSOR GENE NEAR THE D12Mit279 REGION.

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Loss of heterozygosity(LOH) analysis is a standard method to localize a region harboring tumor suppressor gene. Our previous analysis of gamma ray-induced mouse thymic lymphomas revealed three loci exhibiting frequent allelic losses in the vicinities of *D11Mit74, D12Mit279* and *D16Mit122*. This paper shows the fine physical mapping of one locus (*TLSR12a*) around *D12Mit279* and describes several candidates of a novel tumor suppressor gene. For this analysis, a total of 550 lymphomas and 149 subcutaneous tumors were induced in F1 and N2 mice between BALB/c and MSM strains belonging to two different subspecies. Construction of a physical map of *TLSR12a* and LOH analysis using YAC- and BAC-end polymorphic probes localized a region showing the highest LOH frequency in four overlapping BACs. Digestion of the clones with *Not*I produced ten DNA fragments from 15 kb to 140 kb, one 20 kb fragment of which comprised the LOH-peak region. This suggests the presence of a tumor suppressor gene in the fragment or around the *Not*I site. Accordingly, genes and exons have been screened by sequencing, analysis by the GRAIL program, and data base comparison. We discuss properties of those candidates and possible function as a novel tumor suppressor gene.

B28. HOTFOOT MOUSE MUTATIONS AFFECT THE δ2 GLUTAMATE RECEPTOR GENE AND ARE ALLELIC TO LURCHER.

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Hotfoot (*ho*) is a recessive mouse mutation characterized by cerebellar ataxia associated with relatively mild abnormalities of the cerebellum. It has been previously mapped to Chromosome 6 and at least eight independent alleles have been reported. In order to clone the gene responsible for the hotfoot phenotype, we use two complementary strategies: the cloning of the insertion site of a transgene (in the $ho^{TgN37INRA}$ allele) and a classical positional cloning approach.

A short genomic sequence flanking the transgene insertion was cloned and used to screen a BAC library. A 18 kb physical map around the wild type counterpart of the insertion site was established and sequenced. Exon trapping strategy did not identify any relevant candidate sequences for the hotfoot gene in these 18 kb nor in the entire BAC.

Alternatively, we mapped the *hotfoot* locus in a 1.1 cM region between *D6Mit122* and *D6Mit174* microsatellite markers. Another mouse mutation, Lurcher (*Lc*) had been mapped to this genetic region (Zuo *et al.* (1995) *Genome Res* 5: 381-392.) and recently found to be due to a mutation in the glutamate receptor ionotropic δ2 gene (*Grid2*) (Zuo et al. (1997) *Nature* 388: 769-773). Since Grid2 was an interesting candidate gene for the hotfoot mutation, its coding sequence was analysed in three independent alleles of *hotfoot*: ho^{4J}, $ho^{TgN37INRA}$ and ho^{Nancy} (Guastavino et al. (1990) *Brain Res.* 523: 199-210). Deletions of 510 bp and 948 bp were found in the *Grid2* coding sequence in the ho^{4J} and ho^{Nancy} alleles respectively. Analysis of the $ho^{TgN37INRA}$ allele revealed a chimeric mRNA between the *Grid2* gene and sequences of the transgene.

The exceptionally high number of independent alleles at the hotfoot (*ho*) locus is an invaluable tool for investigating the function of the Glutamate Receptor Ionotropic δ2 protein which so far remains largely unknown.

B29. MOUSE MODELS FOR VELOCARDIOFACIAL SYNDROME BASED ON COMPARATIVE PHYSICAL MAPS, SEQUENCE ANALYSIS, AND CHROMOSOME ENGINEERING.

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The region of human 22q11.2 commonly deleted in Velocardiofacial Syndrome (VCFS) and related developmental syndromes includes a region of 1.4 Mb between *IDD* and *RanBP1*. This area has been the focus of exhaustive gene discovery and complete sequencing. No single gene has been shown to cause VCFS, suggesting that it is a caused by the combined effects of the loss of several genes in the region. In the commonly deleted region, 28 transcripts have been reported, but the function of most of these genes and their possible roles in the etiology of VCFS are unknown. Expression studies and targeted deletions of candidate genes or chromosomal segments can be undertaken in the mouse. To provide the necessary reagents, a YAC/BAC/PAC contig has been constructed spanning 5-6 MB of mouse Chr 16, and a detailed map prepared for the 2.5 MB region corresponding to the VCFS region. YAC fragmentation was used to determine physical distances between 33 (of 54) markers in this region. With the exception of *CLTCL*, which is absent from the mouse, the genes from the region of human 22q11.2 from *Idd* to *N41* are found in two adjacent regions of conserved synteny on mouse Chr. 16.

Eight BAC or PAC clones covering over 900 kb are in the contig closure stage of sequencing. Sequencing efforts to date have produced 220 kb of completed mouse genomic sequence from two overlapping BACs, including a site of chromosomal rearrangement between mouse and human. This mouse sequence is being analyzed using GRAIL and BLAST searches of established databases with custom automated scripts, and by direct comparison to the sequence of the corresponding human region using a dot plot program written to handle comparisons of large genomic regions. 100 kb of mouse sequence between *Idd* and *Ctp* corresponds to 140 kb of human sequence. Five novel transcripts are present in the mouse sequence. Comparison of the corresponding mouse and human sequence reveals differences in gene spacing, function, and the absence from mouse of several reported human genes. Regions of unknown function that have been conserved during the 80 million years since the divergence of the mammals but which don't appear to be genes or promoters have been identified. Studies into the genetic basis of developmental anomalies in VCFS have been initiated using targeted homologous recombination to create conventional and Cre/*lox*P knock outs spanning the region. Mice with a targeted deletion of *Gscl* have been generated and are being analyzed for craniofacial abnormalities.

B30. INITIATION OF THE U.S. MOUSE PHYSICAL MAPPING CONSORTIUM.

John D. McPherson *and the U.S. Mouse Mapping Consortium.*

A collaborative effort between four laboratories: J. McPherson, M. Marra and R. Waterston , Washington University School of Medicine; E. Lander and B. Birren, Whitehead Institute for Biomedical Research; P. de Jong, Roswell Park Cancer Institute; and R. Kucherlapati, Albert Einstein College of Medicine has been initiated to begin to generate a bacterial artificial chromosome (BAC) physical map of the mouse genome. Expressed sequence tag (EST) markers from the newly founded U.S. mouse radiation hybrid mapping effort (*T.J. Hudson and E. Lander, Whitehead Institute and MIT) are used to isolate BAC clones from the RPCI-22 mouse library (129/SvEvTACfBr). In addition, previously mapped genetic markers and genes will be used as probes. The isolated clones are then assembled into contigs using restriction fragment fingerprinting methods. A database of these anchored contigs will be accessible via the internet. The goals and progress of this effort will be presented.

B31. SKELETAL MUSCLE IN THE MYODYSTROPHY MOUSE SHOWS SARCOLEMMAL DISRUPTION.

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Myodystrophy (myd) is a spontaneous, autosomal recessive mouse mutant characterized by impaired growth, progressive weakness, dystrophic muscle histology, elevated serum creatine kinase, sensorineural hearing loss and premature death. It maps to the midregion of mouse chromosome 8, which has conserved synteny with human chromosome 4q31-35. We have proposed the mutation as an animal model of facioscapulohumeral dystrophy (FSHD), an autosomal dominant muscular dystrophy that maps to human 4q35, but for which no gene has been isolated. We are working to positionally clone the myd gene, which would be an attractive candidate for FSHD or some other human dystrophy, and in addition, we are undertaking a more detailed characterization of the myd pathophysiology. The elevated CK in affected animals suggests an increased sarcolemmal membrane permeability. To confimthe altered membrane permeability, and to study the pattern of muscle damage, we injected myd mice with Evans blue dye (EBD), an inert dye that is impermeable to intact cellular membranes. EBD uptake was seen in skeletal muscles throughout the bodies of myd animals. Cardiac muscle was spared. The results are compared to those reported for two other dystrophic mouse mutants. In contrast to the myd results presented here, mdx muscle shows EBD staining of cardiac muscle and preferential staining of limb girdle muscles, while dy and dy2J muscles do not show EBD uptake (Straub et al., 1997). These results indicate that the myd mouse has ongoing loss of muscle membrane integrity, and is more similar in this respect to the dystrophin-deficient mdx mouse than the laminin alpha-2 (merosin)-deficient dy and dy2J mice.

B32. IDENTIFICATION OF MOUSE TRANSCRIBED SEQUENCES BY DIRECT cDNA SELECTION: AN INTERSPERSED REPETITIVE ELEMENT-PCR (IRE-PCR) BASED NOVEL APPROACH.

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Direct cDNA selection is well established as an effective method for identifying gene sequences encoded by critical region genomic clones. A significant limitation, however, of the application of direct cDNA selection to positional cloning projects has been the requirement of isolation of uncontaminated genomic template DNA. This typically involves purification of YAC DNA by preparative pulsed field gel electrophoresis (PFGE), and is frequently complicated by inadequate resolution of YAC and host yeast chromosomes, resulting in gross contamination of direct selected clones with rRNA. Biotin labeling of the template, required for fluid-phase selection with magnetic separation, is also inefficient, and requires monitoring by use of radioactively labeled nucleotide, during nick translation.

We have used B1 and B2 IRE-PCR, with biotin-labeled repetitive element primers, as a one-step method for isolating and biotin labeling template for direct cDNA selection. This method selectively amplifies and biotinylates, regions of genomic DNA flanking mouse B1 and B2 repetitive elements from critical region YACs/BACs etc. Amplified product is then used as the template for direct selection. The advantages of this technique are: (1) purification of YAC DNA by PFGE is not required since yeast or *E. coli* do not contain B1 or B2 repetitive elements, (2) use of 5'-biotin labeled B1/B2 primers ensures efficient generation of biotinylated fragments of genomic DNA by PCR, and (3) isolation of novel STSs flanking B1/B2 transcribed sequences that may be useful for saturating a genomic region with novel genetic markers (we have recently reported that these regions are enriched for polymorphic simple sequence repeats). Since B1 and B2 repetitive elements are abundant within the mouse genome, this approach results in relatively little loss of template complexity. Genomic coverage is optimized by long-range PCR techniques.

Using this method, we have isolated transcribed sequences from YACs/BACs within the critical regions for the mouse mutations *sdy, mu* and *gm*. Direct selected cDNAs from all 3 regions, that mapped back to the appropriate critical region, were isolated after two cycles of enrichment. These cDNA clones represent potential candidate genes for the 3 mutations and are being further evaluated. While the success of this method depends on the presence of mouse B1 or B2 elements adjacent to or within genes, it still is a very convenient and rapid approach to identifying some genes in the genomic interval of interest. This method is useful not only for positional cloning projects, but also for rapidly generating regional transcription unit maps. The method is applicable to other mammalian species by utilizing other high-copy, species-specific repetitive elements. about the mechanisms by which the streak assembles and adopts these regional specific functions.

B33. THE MOUSE Y CHROMOSOME INTERVAL NECESSARY FOR SPERMATOGONIAL PROLIFERATION IS GENE DENSE WITH SYNTENIC HOMOLOGY TO THE HUMAN AZFa REGION.

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The Δ*Sxrb* deletion interval of the mouse Y chromosome contains *Spy*, a spermatogenesis factor gene(s) whose expression is essential for the post-natal development of the mitotic germ cells: spermatogonia. The boundaries of Δ*Sxrb* are defined by the duplicated genes *Zfy1* and *Zfy2*, and four further genes have previously been mapped within the interval : *Ube1y* and *Smcy*, linked with *Zfy1* on a contig of 250 kb, and *Dffry* and *Uty*, which were unanchored. The interval was estimated to be >450 kb. In order to identify further gene(s) that may underlie *Spy*, systematic exon-trapping was performed on an extended contig, anchored on *Zfy1*, which covers 750 kb of the ΔSxrb interval. Exons from two novel genes were isolated and placed together with *Dffry* and *Uty* on the contig in the order, *Dffry - Dby - Uty - Tspy - Eif2gy - Smcy - Ube1y - Zfy1*. All the genes, with the double exception of *Tspy*, are X-Y homologous and produce putatively functional, spliced transcripts. The tight linkage and order of *Dffry, Dby* and *Uty* was shown to be conserved in deletion intervals 5C/5D of the human Y chromosome by the construction of a contig of human PAC and YAC clones; and this represents the first example of syntenic homology between Y chromosomes from two distinct mammalian orders. Interval 5C/5D contains the distal boundary of the *AZFa* interval which, like Δ*Sxrb*, is believed to be necessary for spermatogonial development in the prepubertal testis. Our results therefore show that *AZFa* and *Spy* may be encoded by homologous genes.

B34. CELL CYCLE REGULATION IN PLASMA CELLS BY ALLELIC VARIANTS OF THE CDKN2A LOCUS, A CANDIDATE GENE FOR PCTR1.

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Mouse plasma cell tumors are a model for several human B cell malignancies. BALB/c mice are susceptible to tumor induction, whereas DBA/2 mice are resistant. Three susceptibility loci, *Pctr1-3*, linked to mouse Chr 4 have been shown to determine susceptibility/resistance patterns in backcross mice. The *Cdkn2a* (p16, p19) locus was mapped to the same interval as*Pctr1* and became a candidate gene for this tumor susceptibility locus. Upon sequencing Cdkn2a cDNA, two variants were found in BALB/c mice; the proteins were inefficient in their ability to inhibit the activity of cyclin D2/CDK4 in kinase assays with RB protein. Two plasmacytoma lines were transiently transfected with plasmids expressing wild-type (DBA/2) p16 and two sequence variants specific to BALB/c mice. Forty-eight hours after transfection, DNA content was assayed by flow cytometry to determine cell cycle distribution, with gating to restrict analysis to cells expressing a co-transfected marker, membrane-targeted GFP. Overexpression of wild-type p16 led to G1 cell cycle arrest, however, the two allelic variants specific to BALB/c were similar to control transfections with empty vector. The transfection experiments support the in vitro kinase assays and continue to suggest that the inherited allele plays an important role in the genetic susceptibility of BALB/c mice for plasmacytoma induction.

B35. CDNA NUCLEOTIDE SEQUENCE OF A NEW MOUSE HBB HAPLOTYPE W1 AND ITS EVOLUTIONARY RELATIONSHIP WITH D- AND P- HAPLOTYPE HBB GENES.

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In east Asia including mainland China north of Yangtze River, Korea and Japan, a house mouse subspecies, Mus musculus musculus, and some other genetically close ones inhabit. We call them musculus subspecies group. In those regions, p-haplotype Hbb is frequently observed. In China south of Yangtze River and southeast Asia such as the Philippines, Taiwan and Indonesia, castaneus subspecies group of mice which includes at least two types of Mus musculus castaneus inhabit. In these regions, d-haplotype Hbb is predominant.

Recently we have found a novel Hbb haplotype w1 which is distributed in northwestern region of China with the higher frequency (Kawashima et al, 1991). In this study, nucleotide sequences of cDNAs in the two hemoglobin genes of w1 haplotype on chromosome 7, b1-w1 and b2-w1, are analyzed and compared with those of d- and p-haplotype, respectively. The sequences of b1-d and b1-p are quite similar, and those of b2-w1 and b2-p as well. On the other hand, several nucleotide substitutions are detected between b1-d and b1-w1 and also between b2-d and b2-w1. These findings suggest a possibility that probably after differentiation of Mus musculus subspecies, an intersubspecies hybridization occurred between d-haplotype and w1-haplotype Hbb chromosomes, in which a meiotic recombination should have occurred later and p-haplotype with b1-d and b2-w1 genes has emerged. Thus, p-haplotype could be an inter-subspecies recombinant. Geographically p-haplotype is just distributed in the intermediate region between w1- and d- regions in China which can support the above possibility as well.

Reference: Kawashima, T., Miyashita, N., Tsuchiya, K., Li-H., Wang-FS., Wang-CH., Wu-XL.,Wang-CY., Jin-ML., He-XQ., Kryukov, A., Yakimenko, L.V., Frisman, L.V., Moriwaki, K. (1995) Geographical distribution of the Hbb haplotypes in the Mus musculus subspecies in eastern Asia. Jap. J.Genet. 70:17-23.

B36. ANOMALOUS (PREDUODENAL) PORTAL VEIN; AN AUTOSOMAL RECESSIVE MUTATION IN MICE.

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The heritable portal vein anomaly i.e., preduodenal portal vein (pdpv) was found in the AKR/J strain of mice. Usually, the portal vein runs at the dorsal side of the duodenum. In this mutant, however, it was found in the ventral side of the duodenum. The "pdpv" was not found in 54 inbred mouse strains in our mouse colony. This anomaly is known in human as a rare congenital anomaly. It is found sporadically by epigastric pain or other symptoms such as vomiting, jaundice, etc. This anomaly has a high association with other congenital anomalies (malrotation, situs inversus, pancreatic anomalies, duodenal anomalies, biliary system anomalies, splenic anomalies, dextrocardia, etc.), although the inheritance of this anomaly has not been examined. In contrast, no symptoms and no combination of other congenital anomalies were observed in the "pdpv" mice.

The "pdpv" mice were mated with tester stock of mouse strain PT which has 7 recessive marker genes on the chromosomes 2, 4, 7, 9 and 14. The F1 animals were normal, irrespective of the sex of mated parents. F1 mice were back-crossed with AKR/J and "pdpv" was found in 89 of 183 pups (48.6%). The segregation ratio of abnormal and normal portal vein was 1:1, indicating that "pdpv" is determined by a single autosomal recessive gene. Gene causing "pdpv" was not linked to chromosome 7.

B37. MOLECULAR CHARACTERIZATION OF *Tlhm1*, A NOVEL GENE EXPRESSED IN MURINE HEMATOPOIETIC TISSUE.

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A collection of cDNA clones from the peri-implantation stage of mouse embryogenesis includes a full-length novel cDNA that is primarily expressed in all hematopoietic tissues and lineages. It is designated Tri-Lineage Hematopoietic Marker (*Tlhm1*), reflecting its mode of expression. Northern analysis using staged mouse embryo and mouse multiple organ blots reveals a single 1.8 kb transcript, present at a very low level at 7 d.p.c., and increasing in abundance through 17 d.p.c. Of the adult mouse organs tested, the Tlhm1 transcript is only detected in the spleen. More sensitive analysis using RT-PCR and total RNA extracted from the organs of mouse embryos at various stages showed Tlhm1 expression at developmentally regulated sites of hematopoietic activity -- specifically, the aorta-gonad-mesonephros region of the 9.5 d.p.c. embryo, and an intraembryonic site of the 13.5 d.p.c. embryo. Preliminary in situ hybridization results in the 7.5 d.p.c. embryo show a low level of Tlhm1 expression within the embryo and strong expression in the chorion and in the region of the blood island. In the 13.5 d.p.c. embryo strong expression is shown in a small number of cells scattered throughout the liver. At later stages of development, RT-PCR shows prominent Tlhm1 expression in the liver of the neonate mouse, and in the spleen, thymus, and bone marrow of both the neonate and adult mouse. To determine if Tlhm1 is expressed in lineage-committed hematopoietic cells, we carried out RT-PCR on total RNA from mouse cell lines derived from the three hematopoietic lineages. Lymphoid expression was demonstrated in the B cell line P3X63, T cell lines EL-4, YAC, and natural killer cells purified from neonate B6 liver. Tlhm1 expression was also demonstrated in P815, a mastocytoma cell line representing the myeloid lineage, and MEL cells, an erythroid cell line.

Conceptual translation of the 1538 bp Tlhm1 cDNA predicts a peptide of 226 amino acids, with a molecular weight of 25 kDa. Hydrophobicity profiles of the predicted peptide reveal four potential transmembrane regions. A Blastp search demonstrates similarity at the amino acid level with the tetraspanin family of cell surface proteins. We are presently characterizing Tlhm1 expression in adult mouse bone marrow cells isolated by FACS, in order to determine expression in multipotent hematopoietic progenitors and subpopulations of more mature lineage committed hematopoietic progenitors. The Tlhm1 human ortholog has also been recovered, and its cDNA sequence, genomic structure, and expression patterns are being determined.

The mode of Tlhm1 expression and its predicted membrane topology make it a strong candidate marker for the study of hematopoiesis and for clinical analysis.

Around 1 in every 100 progeny are carrying heritable dominant mutations and examples of novel mutations and phenotypes that have been identified will be reported.

B38. MOLECULAR CHARACTERIZATION OF DELETIONS ON MOUSE CHROMOSOME 11 CONTRIBUTING TO α-THALLASEMIA AND PERIMPLANTATION LETHALITY.

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Alpha thallasemia is a hereditary hematopoetic disorder due to deletions or mutations at the a-globin locus. Humans who are heterozygous for a-thallasemia display a microcystic, hypochromic anemia. Homozygous disruption of the α-globin locus is lethal between 30-40 weeks of gestation. While there are no naturally occurring animal model for α-thallasemia, three strains of a-thallasimic mice with deletions at the α-globin locus (*Hba2*) have been generated using X-irradiation or chemical (triethylenemelamine) induction. In addition to the α-thallasemic phenotype seen in the heterozygous state, these deletion strains also demonstrate perimplantation lethality when bred to homozygosity. This lethality occurs at a much earlier timepoint than in human gestation, indicative of a nearby recessive gene(s) being disrupted. Since hemoglobin is not expressed until embryonic day 8.0 and these mice die before then, the α-globin is not responsible for this lethal phenotype. Molecular characterization of the deletion strains using SSLP markers around the α-globin locus determined that the *Hba2*$^{(th)j}$ deletion is only missing *D11Mit53* (part of the α-globin locus). The other two strains are missing additional SSLP loci indicating they have larger deletions. Further characterization of the deletion region by contig analysis will help define the extent of the various deletions. This will also aid in estimation of the number of genes within the region which may be contributing to the perimplantation lethality.

B39. THE MOUSE MUTANT *mceph*, WITH ENLARGED BRAIN, HAS DISTURBANCES IN THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM.

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Megencephaly, enlarged brain, is the major sign in several human neurological diseases and syndromes. The mouse model for megencephaly, *mceph*, has an enlarged brain and a lowered body weight. It displays various morphological, neurological and motoric disturbances. Previous studies have suggested that the enlargement of the brain is due to hypertrophy of the brain cells, rather than an increased number of cells. No structural abnormalities have been found. In this study we have by *in situ* hybridization, investigated the mRNA expression pattern of the Insulin-like Growth Factor (IGF) system in the brains of 9-10 weeks old mutant *mceph* mice compared to controls. ^{35}S labeled synthetic oligonucleotide probes for IGF I, IGF II, Type I IGF receptor (R), Type II IGF R, IGF Binding Protein (BP)-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6 and a regulating hormone Transforming Growth Factor (TGF)β1 were used to determine the mRNA expression. In the mutants there was an increased expression level of IGFBP-4, IGFBP-6 and TGFb1 compared to normal mice, whereas the difference in expression level of IGFBP-5 between mutants and controls was more complex. The marked increase of IGFBP-4 was found in two cortex cell layers, extending from the frontal to the dorsal part of the cerebrum. IGFBP-5 showed a decreased mRNA level specifically in the dentate gyrus of the hippocampus, whereas the expression was increased in different cortical layers. The substantial increase of IGFBP-6 was found in the dentate gyrus. TGFb1 was increased in the lower dorsal parts of the cortex. In the liver, there was no difference in the expression of any of these mRNA species between *mceph* mice and controls. These data strongly suggest an important role for the IGF system in the growth regulation of the brain of the *mceph* mouse.

B40. GLUTATHIONE REDUCTASE ACTIVITY DEFICIENCY IN *Mus musculus*: A MODEL FOR THE HUMAN DISEASE.

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In man, deficiency of erythrocyte glutathione reductase (GR) has been reported in association with diverse clinical syndromes, which may include haemolysis and neurological disease. It is unclear whether these syndromes represent specific genetic entities or, as it has been shown that GR deficiency can be due to deficiency of the cofactor flavin adenine dinucleotide, the symptoms are a result of the GR deficiency in combination with a dietary deficiency of riboflavin.

We have recently recovered the first mouse GR mutant with approximately 50 % residual enzyme activity in blood compared to wild type among offspring of mutagen-treated male mice. The reduction of GR activity was evident in both blood and in other tissue extracts. Homozygous mutants with only 2 % residual enzyme activity were recovered in progeny of *inter se* matings of heterozygotes. Results of linkage studies indicate a mutation at the *Gr1* structural locus on chromosome 8; the deduced map position is: Centromere - *D8Mit335* - (8.3±2.8) - *Gr1*^{a1Neu} - (1.0±1.0) - *D8Mit190* - (16.7±3.8) - *D8Mit305* (distances in cM). Routine haematological tests were performed to determine the possible effect of the GR deficiency on erythrocyte metabolism and to exclude the possibility that the reduced GR activity in blood results indirectly from altered erythrocyte dynamics. The results obtained indicate that the reduction of GR activity is not a result of an alteration in haematopoiesis. Other physiological traits, such as body weight and organo-somatic indices of liver, lung, kidney, spleen and heart also indicated no differences between wild types and mutants. These findings demonstrate that the mutation in either the heterozygous or in the homozygous state does not affect physiological functions. There is no evidence of haemolytic anaemia in homozygous GR deficient mice; mutant animals presented neither altered haematological parameters nor the characteristic enlargement of the spleen.

A similar observation has been made in humans in which a family has been described with an almost total deficiency of GR which was not associated with chronic haemolytic anaemia. Based on these results we suggest the mechanism for haemolytic anaemia associated with GR deficiency to be due to the synergistic effect of the enzyme deficiency with a dietary deficiency of riboflavin. The presently described mouse mutation will be useful in testing this hypothesis.

B41. MUTATIONS IN THE UNCONVENTIONAL MYOSIN *Myo15* CAUSE DEAFNESS IN *shaker-2* and *shaker-2^J* MICE, MODELS FOR THE HUMAN NONSYNDROMIC DEAFNESS DFNB3.

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shaker-2 homozygotes show profound hearing loss as well as head-tossing and circling behaviors. Two alleles of *shaker-2* (*sh2* and *sh2^J*) map to a region of chromosome 11 that shows conserved synteny with human chromosome 17p11.2, the location of the nonsyndromic deafness locus DFNB3 (Liang et al. 1998 Am J Hum Genet 62 904). A 140 kb mouse BAC transgene corrects the deafness and circling in *sh2* mice. DNA sequencing of this BAC revealed an unconventional myosin *Myo15*. Myosins are mechanoenzymes that hydrolyze ATP to produce force. A partial *Myo15* cDNA with 50 exons was characterized and a missense mutation was detected in *sh2* mice at a highly conserved cysteine within the motor domain of *Myo15* (Probst et al. 1998 Science 280: 1444). Three mutations in the human homolog of this gene, *MYO15*, co-segregate with congenital, recessive, nonsyndromic deafness in three large unrelated families (Wang et al. 1998 Science 280: 1447). Beyond the previously reported exon 50, an additional 13 exons and a poly (A)+ tail have been identified. Analysis of *shaker-2^J* DNA indicates that the last 5 exons from the 3′ end of *Myo15* are deleted. Auditory brainstem response analyses, scanning electron microscopy, and laser scanning confocal microscopy of *sh2* and *sh2^J* mice indicate that the phenotypes are nearly indistinguishable. Alternative splicing of exon 23 predicts a short and long isoform of *Myo15* with the short form lacking most of the tail region. The *sh2* mutation is in the motor domain of *Myo15* and probably affects both the short and long isoforms, while the *sh2^J* mutation should only affect the long isoform of Myo15. We conclude that the long isoform of Myo15 is critical for the development and maintenance of normal hearing.

This work was supported by the National Institute of Child Health and Human Development R01 HD30428 (SAC), NIH-Deafness and Other Communication Disorders (NIDCD) extramural grants 2PO1 DC00078 and 2RO1 DC 01634 (YR), the National Science Foundation (FJP), the NIGMS T32 GM07863 (FJP), a University of Michigan Rackham Graduate Fellowship (FJP), P01 DC 02982 (DD) and NIDCD Z01 DC 00038-01 (TBF, BF, AW, YL and K N-T).

B42. *PAX6* ^{4NEU}: A HYPOMORPH ALLELE IN THE MOUSE DUE TO AN AMINO ACID SUBSTITUTION IN THE HOMEOBOX DOMAIN.

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Mouse mutations with eye abnormalities provide the opportunity to analyse the function of different genes implied in eye development and physiology and serve as models for human hereditary diseases. A group of six dominant mutations recovered in ethylnitrosourea mutagenesis experiments have been mapped to Chromosome 2 close to the position of the *Pax6* gene. The RNA/DNA sequencing analyses revealed a base pair insertion for one and nucleotide substitutions for the remaining five mutations. The mutations have been detected in the conserved DNA-binding domains, paired box and homeobox and in the Pro/Ser/Thr rich region. Five mutations lead to premature truncation of the protein and the associated phenotype (microphthalmia, iris anomaly and anterior polar cataract with corneal adhesions in heterozygotes, anophthalmia and craniofacial abnormalities with the lack of development of the olfactory cavities in homozygotes) is consistent with the hypothesis that these mutations result in loss of gene function. In contrast homozygotes of *Pax6^{4Neu}* express vestigial, pigmented eyes suggesting that this mutation retains partial gene function. Molecular characterization showed a base pair substitution resulting in a Ser to Pro amino acid change at position 9 in the DNA recognition helix of the homeobox. In vitro mutagenesis experiments have shown that Ser 9 is very important for the specificity of the DNA-binding function of the homeobox. We used a homology modelling procedure to predict the conformational modifications in the mutated homeobox. Results suggest that the Ser to Pro change severely alters the recognition helix. Since we expected that the DNA binding activity of the homeobox was altered, we expressed the mouse wild-type and the *Pax6^{4Neu}* mutant homeobox as fusion proteins in bacterial cells and we tested their ability to bind to a DNA consensus sequence in a gel mobility shift assay. We observed, indeed, that the activity of the mutated homeobox was severely reduced. Mutations in the PAX6 gene (from base substitutions, small deletions/insertions to large deletions/insertions/rearrangements) have been reported in a growing number of aniridia patients, patients with WAGR syndrome and Peter's anomaly (Human PAX6 Mutation Database). The characterization of *Pax6* mutations in the mouse provides valuable animal systems to study the function of the *Pax6* gene.

B43. THREE GENE NEIGHBORS IN 40 kb OF THE MOUSE t-COMPLEX -- TWO NEW GENES (Nbps and D17Wsu15e) AND ONE KNOWN (Als) -- SHOW HEMIZYGOUS DNA METHYLATION PATTERNS.

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While mapping ESTs for new cDNA clones from the ectoplacental cone of 7.5-dpc mouse embryos, we found unusual clustering in the t-complex on Chromosome 17. Subsequent screening with the ESTs revealed that two genes, *D17Wsu11e* (Nbps; see Grahovac et al., these Abstracts) and *D17Wsu15e*, were located on a single BAC clone. Genomic Southern blot analysis showed that the distance between these genes was within 30 kb, an observation confirmed by direct genomic sequencing (Kargul et al., these Abstracts). The BAC clone was partially digested with *Sau3*AI, subcloned into a vector, and 384 subclones were arrayed in 96-well microtiter plates. The mini-library was screened with Nbps and *D17Wsu15e* cDNAs as probes. Positive clones were partially sequenced, and revealed another gene, Acid Labile Subunit of Insulin-like Growth Factor Binding Complex (*Als*), very near the *Nbps* gene. This gene binds and stabilizes the IGF/IGFBP3 complex in serum to extend its half-life, and is thought to be involved in the control of IGF function.

Since an imprinted region of the t-complex is nearby, we tested whether the *Nbps*, *Als*, and *D17Wsu15e* genes are imprinted, using the 50% methylation assay. Southern blotting was performed on genomic DNA isolated from the spleens of 129SV/J mice with *Nbps*, *Als*, and *D17Wsu15e* cDNA as probes. Each of the three genes showed at least one hemizygously methylated CpG site, suggesting genomic imprinting. We further analyzed the methylation sites in the 175 kb BAC clone sequence (Kargul et al., these Abstracts). Genomic sequence allowed us to pinpoint the *HpaII/MspI* sites that showed hemizygous DNA methylation, as well as clarifying the entire exon-intron structures and relative locations of the three genes.

To examine further the possibility of genomic imprinting of these genes, allele-specific gene expression analyses in the interspecific F1 hybrid mouse using regular and a single-cell RT-PCR are underway. The extent of this apparent region of hemizygous methylation will discussed in relation to mechanisms of imprinting and t-complex function.

B44. LINKAGE ANALYSIS OF THE GUTTER SHAPED ROOT (*GSR*) ON MOUSE CHROMOSOME 5.

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In human, fused roots of the second molar (M2) and of the third molar (M2) in lower jaw are called a Gutter Shaped Root (GSR). Many investigators have shown that there are differences in the incidence of GSR among races. It is said that the appearance of the GSR is predominantly influenced by a genetic factor(s), rather than by environmental factors, but the cause of the GSR formation is not known. Recently, we reported that C57L/J mice were useful models for studying the cause of the appearance of GSR; they had about 90-100% incidence rates of GSR formation and there were no significant differences between the sexes. We performed the genetic crosses using two strains of mice, C57L/J with GSR and C57BL/6J mice with normal roots. It was suggested that a few genes would be playing a role in inducing the GSR. Based on our results from the genetic crosses, we searched for a candidate chromosome using pooled DNA from affected backcross mice, (C57L/J x C57BL/6J) F1 X C57BL/6J. We identified some candidate chromosomes, Chr5, 8, 15, 17 and 19, for the development of the GSR. Particularly, *Msx1*, *Fgfr3*, *Bmp3* and *Pdgfa* have been mapped on mouse chromosome 5 suggesting relation to tooth development. The purpose of present study was to identify a candidate gene causing the GSR on mouse chromosome 5. Therefore, we performed linkage analysis on mouse chromosome 5. Thirteen polymorphic microsatellite markers were detected on chromosome 5 and performed the genotyping of the 25 backcross mice with GSR. Single marker analysis determined high linkage at *D5Mit161, 29, 321, 427* and *166* (chi-square=6.76, $p<0.01$). Based on these results, there was a possibility that *Bmp3* and *Pdgfa* might be candidate genes causing the GSR because the two genes had been mapped near those Mit markers.

B45. CHARACTERISATION OF GENES ISOLATED FROM THE BROWN DELETION COMPLEX.

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The specific locus mutagenesis test generated thirty overlapping deletions at the brown (Tryp1) locus spanning 7-9cM. Complementation analysis revealed four new functional units in the central to distal part of this region, two of which are early embryonic lethals. Our analysis has shown that one appears to result in neonatal death. The fourth, baf (brown associated fitness) has a sub-viable phenotype, demonstrating poor growth, gut abnormalities, nervous behaviour and death at around weaning age. This phenotype appears to be highly variable, with some homozygotes surviving to adulthood. We are currently making a comparative study of the different deletions in there homozygous state to determine if these phenotypes or there severity are genetically separable.

We have generated a fine structure map of a 2.5cM region encompassing these genes containing over 30 markers. Techniques including, sample sequencing, exon trapping ,cDNA selection and use of the conserved syntenic region on human chromosome 9 have identified transcripts from this region.. Of particular interest is a transcript containing 13 PDZ domains which maps to the region. PDZ domains are know to interact with a number of proteins at specific junctions and may be responsible for the sub-cellular localisation and clustering of proteins into functional complexes. There is evidence that the protein interacts with the C-terminus of the 5-HT2c receptor. To determine the possible roles of this gene in vitro functional assays, behavioural observations and immunohistochemistry techniques will be utilised. Although the gene is not deleted from some deletions that give rise to baf animals, the proximity of the gene to the critical region means that it cannot be excluded as a baf candidate. Expression studies are currently being undertaken to determine if the quantitative, temporal, or spatial characteristics of expression are altered in these mutant animals.

B46. THE TIGHT SKIN PHENOTYPE OF Tsk/+ MICE IS NOT DEPENDENT ON THE PRESENCE OF MATURE T AND B LYMPHOCYTES.

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The mutant phenotype of Tight skin (*Tsk*) mice, which includes thickened skin and visceral fibrosis, has made *Tsk*/+ mice models for hereditary emphysema, myocardial hypertrophy, scleroderma and other connective tissue abnormalities. We demonstrated that the *Tsk* chromosome has a 30-40 kilobase genomic duplication within the fibrillin1 (*Fbn1*) gene that results in a larger than normal, in-frame *Fbn1* transcript. Dermal fibroblasts from *Tsk*/+ mice synthesized and secreted both normal Fbn1 (~330 kDa) and the mutant oversized *Tsk*-specific *Fbn1* protein (~450 kDa) in comparable amounts, and the Tsk-specific Fbn1 protein was stably incorporated into cell layers. Immunohistochemical and ultrastructural studies of +/+ and Tsk/+ mouse skin demonstrated differences in the organization and distribution of microfibrillar arrays. These data support the production of the predicted mutant Fbn1 glycoprotein and show that it is assembled into a discrete population of abnormal microfibrils with altered molecular organization and aggregation properties. Several previous studies had suggested that the immune system may have a role in generating the cutaneous fibrosis of *Tsk*/+ mice. The Rag2 gene is essential for the generation of mature T and B lymphocytes, since loss of *Rag*2 function results in a total inability to initiate V(D)J rearrangement of immunoglobulin (*Ig*) and T cell receptor (*Tcr*) genes *in vivo*. Therefore, we established mice that are homozygous for the *Rag*2 knockout and heterozygous for the *Tsk* mutation. Genotypic and phenotypic analyses show that the severity of skin tightness of *Tsk*/+ mice is not dependent on the presence of mature T and B cells. In addition, the data further extend our previous findings of a close association between the genomic duplication within the *Fbn1* gene, the secretion and assembly of the mutant Fbn1 glycoprotein into abnormal microfibrils, and the tight skin phenotype.

B47. MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AN IMPRINTED REGION OF MOUSE CHROMOSOME 7 AND RELATED SEGMENTS OF HUMAN CHROMOSOME 19.

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Genetic studies have suggested that portions of proximal Mmu7 might contain genes that are parentally imprinted, and recently, a single paternally expressed gene, *Peg3* has been assigned to this genomic region. We have constructed physical maps of the mouse regions surrounding *Peg3* and the related interval of human chromosome 19q13.4, and have identified and characterized a number of novel genes in both species. In addition to *Peg3*, we have now identified a novel mouse gene, *Zim1* (imprinted Zinc-Finger gene 1) which is expressed exclusively from the maternal allele in most tissues. *Zim1* is expressed at high levels only during embryonic and neonatal stages, and is transcribed at especially high levels in the embryonic heart and the developing nervous system. Although *Zim1* expression is monoallelic in most tissues, transcripts are generated from both parental alleles in neonatal and adult brain. Northern blot analysis has identified four *Zim1* transcripts that range in size from 7.5 kb to 15 kb. The 7.5 kb transcript is expressed at highest levels, and appears to be embryo-specific. We have identified the potential human ortholog of *Zim1* and have mapped the gene near PEG3 in a conserved, gene-rich region of human chromosome 19q13.4. The close juxtaposition of reciprocally imprinted genes is also seen in other well-known imprinted regions, such as human 11p15.5/ Mmu7 (e.g. *H19/Igf2*). These and other recent data suggest the presence of gene-rich, conserved, and unexplored mouse and human imprinted domains in human chromosome 19q13.4 and proximal Mmu7.

B48. IDENTIFICATION OF A DELETION AND A TANDEM DUPLICATION IN ALLELES OF THE PEARL (*Ap3bl*) GENE.

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The pearl (*pe*) mutation on mouse chromosome 13 causes platelet storage pool deficiency, lysosomal abnormalities, pigmentation dilution and decreased retinal sensitivity. It is a member of a series of mouse pigmentation mutations which are models for human Hermansky-Pudlak Syndrome. The βeta3A gene of the AP-3 adaptor complex was identified as a candidate gene for pearl by positional/candidate cloning approaches. βeta3A is an appropriate candidate as the AP-3 complex likely plays a role in protein sorting at the *trans*-Golgi network and in more peripheral regions of the cell. By direct sequencing of βeta3A cDNAs, the *Ap3bl* gene of pe mice contains a tandem internal duplication of 792 bp while in pe^{8J} mice, it has a deletion of 107 bp. Both alterations are predicted to lead to substantial C-terminal truncations of the βeta3A protein and likely to loss of AP-3 functions in pearl. In agreement with this prediction, no mutant βeta3A protein is detectable in pearl tissues by Western blotting. Mutant transcripts are reduced in quantity and predictably altered in size. Also, the subcellular distribution of the AP-3 complex is abnormal in pearl cells. These data indicate the AP-3 complex is a master regulator of several intracellular organelles. In addition, the various pearl mutant phenotypes predict novel functions for the AP-3 complex in mammals.

B49. IDENTIFYING INTERACTING PARTNERS TO THE MOUSE MYOSIN VIIA PROTEIN INVOLVED IN GENETIC DEAFNESS.

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The shaker1 (*Myo7a*) mouse deafness locus is encoded by an unconventional myosin gene - myosin VIIA. The myosin VIIA gene is expressed in hair cells in the cochlea where it is thought to function in development of the neuroepithelium where auditory transduction takes place. In order to understand better the function of myosin VIIA we have investigated the proteins that interact with myosin VIIA. For this purpose we have used two approaches.

Firstly, we have made five myosin fusion proteins covering various regions of the myosin VIIA tail. The myosin VIIA tail has a number of domains with homology to other unconventional myosins, a large region of homology to a tail domain from a plant kinesin, a region of homology to the band 4.1 family as well as an SH3 domain (Mburu et al. 1997 *Genes and Function* 1: 191). Fusion proteins were made using the PinPoint expression vector system, which carries a segment encoding a peptide that becomes biotinylated in E. coli. The biotinylated myosin fusion proteins were purified on avidin columns. Potential myosin interacting proteins were purified from kidney and testis using a column with immobilised myosin fusion protein corresponding to the myosin VIIA SH3 domain. The eluted material consisted of three electrophoretic species with MW of 50, 70 and 100 kD.

As an alternative approach to the identification of myosin interacting partners we have used specific anti-myosin VIIA polyclonal antibodies. Those antibodies were raised against peptides corresponding to different regions of the myosin VIIA tail. These anti-myosin antibodies were used for preparation of affinity columns. Immunoreactive material from kidney and testis was eluted from anti-myosin affinity columns and consisted of 4 peptides with molecular weight of 46, 55, 70 and 150 kD.

The identity of the peptides purified by both approaches was subsequently analysed by N-terminal protein sequencing. A potential myosin VIIA interacting partner has been identified and further characterisation is underway.

B50. POSITIONAL CLONING AND MOLECULAR ANALYSIS OF THE MOUSE DEAFNESS MUTATION (*wi*).

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Genetically determined deafness accounts for more than 50% of all severe childhood deafness and has an incidence of 1 in 2000 live births. Of those with genetic deafness, two thirds suffer from an autosomal recessive pattern of inheritance that is non-syndromic and neuroepithelial in origin resulting from defects in the development and function of the organ of Corti - the site of auditory transduction Whirler (*wi*) is an autosomal recessive mutation that causes deafness and vestibular dysfunction in adult mice that is manifested by head-tossing and circling behaviour and is also neuroepithelial in origin. It has been mapped by linkage analysis to mouse chromosome 4. High resolution genetic maps in the vicinity of the *wi* locus have been constructed using large interspecific and intraspecific backcrosses segregating the *wi* mutation. *wi* is flanked by the markers *Ambp/D4Mit87* and *Tcn* mapping 0.13cM proximal and 0.39cM distal respectively of *wi*. The gene Orosmucoid (*Orm1*) has been shown to be non-recombinant with *wi*.

Using *Orm1* as a start point, a YAC physical map of the whirler non-recombinant region has been constructed. *Orm1* and markers flanking the mutation have successfully been used to screen YAC libraries. Positive YACs have been sized and further STSs isolated from them in the form of end clones (generated using techniques such as TAIL and Vectorette PCR) and new internal STSs (generated by B1 IRS PCR). New STSs have been genetically and physically mapped using Single Stranded Conformational Polymorphism (SSCP) analysis, thereby integrating the genetic and physical map around *wi*. The completion of the YAC contig and its associated STSs has provided a robust framework for the construction of a detailed BAC contig across the wi non-recombinant region. 3 BACs complete a minimal tiling pathway which completely encompasses the wi non-recombinant region and contains the *wi* gene. The BACs have been used to isolate candidate genes for the *wi* mutation using a combination of exon trapping, cDNA selection and sample sequencing. To date both the exon trapping and cDNA selection has produced a large number of clones which are currently undergoing further analysis including expression studies. Several clones detect a novel gene that represents an intriguing candidate for the wi locus. To further narrow and elucidate the region containing the *wi* gene, the 3 BACs from the minimal tiling pathway are now being used to generate transgenic mice for complementation analysis. To date, founder lines for all of the BACs have been established and test complementation crosses with whirler mice are underway.

B51. A NOVEL TYPE OF MYOSIN, MYOSINXV ENCODED BY THE MOUSE DEAFNESS GENE shaker-2.

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Shaker-2 is an autosomal recessive mutation which is located on mouse chromosome 11. Mice homozygous for shaker-2 suffer from deafness and show shaking waltzing and head tossing. Microscopic analysis revealed morphological changes of the sensory hair cells in the inner ear. Here we show the identification of the shaker-2 gene. The gene encodes a novel type of an unconventional myosin, myosin XV. The G to A transition changing cystein to tyrosin in the conserved actin binding domain was detected in sh-2(B.B.R.C. in press). Another mutant strain of shaker-2 which arose spontaneously in ICR was searched for the mutation in myosin XV gene. A missense mutation in the tail domain was detected. These results indicate that myosin XV is responsible for shaker-2 mutation and critical for normal architecture of sensory hair cells.

B52. EVOLUTION OF A YOUNG MURINE GENE FAMILY BY GENE FUSION AND AMPLIFICATION.

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The Sp100-rs gene family forms a large polymorphic cluster on chromosome 1 of *Mus musculus* and closely related species of the genus *Mus*. In the more distantly related species *M. caroli* and *M. cervicolor*, *Sp100-rs* is absent. These species are considered to represent the original status prior to the formation of the gene cluster. We identified two single-copy genes, the 'nuclear dot' gene *Sp100* and *Cla*, the latter less well characterized so far, as contributors to the formation of *Sp100-rs*. *Sp100* is suspected to be a transcription factor and to be involved in virus defense (1, 2). Both *Sp100* and *Cla* are still present as single-copy genes in *M. musculus* and are located in close proximity distally and proximally, respectively, to the gene cluster. The *Sp100-rs* gene is a fusion product of the 5' part of *Sp100* and the 3' part of *Cla*. The promoter regions of *Sp100* and *Sp100-rs* are almost identical, and both genes are stimulatable by interferon. The function of *Sp100-rs*, however, is still unknown. In our model to reconstruct the formation of the gene cluster, the orginal genes, *Sp100* and *Cla*, have been duplicated prior to the fusion event. One copy of each fused to become *Sp100-rs* which was subsequently amplified. The *Sp100-rs* gene family together with the two founder genes offer the opportunity to study two aspects in the evolution of genomes: formation of novel genes by gene fusion and the evolution of gene families by amplification.

1) Xie et al. 1993. Mol. Cell. Biol. 13: 6170-6179. 2) Korioth et a. 1995. J. Cell Biol. 130: 1-13.

B53. ANTISENSE TRANSCRIPTS OF *Gnas*, AN IMPRINTED GENE ON MOUSE CHROMOSOME 2.

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Gnas encodes the G protein alpha stimulatory subunit (Gs alpha), activating the adenylyl cyclase pathway. We have detected endogenous antisense transcripts (*Sang*) to *Gnas* in adult and new born mouse tissues by Northern analysis, RT-PCR, and *in situ* hybridisation expression studies. Multiple isoforms of Sang were observed in heart, skeletal muscle, liver, and kidney. At least 2 (4.5 & 3kb) of the 3 major *Sang* transcripts are larger than *Gnas* (2.1kb). While *Gnas* is expressed ubiquitously, *Sang* is discretely expressed in the thymus; kidney cortex; olfactory sensing organs (Vomeronasal and serous glands); in the actively dividing cells in the base of the vibrissae, and in the keratinizing layer of the skin epithelium. Further, there appears to be a form of reciprocal regulation in the expression of *Sang* and the intensity of expression of *Gnas*. Products sequenced from *Sang* specific cDNAs have novel 5′ and 3′ sequence, encode a 318aa ORF, and overlap the entire coding sequence of *Gnas*. The potential for long, perfectly duplexed *Sang:Gnas* mRNAs suggest a role for *Sang* in regulating translation of *Gnas*. The unusual abundance of multiple small isoforms in the kidney may implicate *Sang* in the subtle imprinting of *Gnas* in the glomerular tufts of new-born mice.

B54. IDENTIFICATION OF IMPRINTED GENES ON DISTAL MOUSE CHROMOSOME 2 BY SUPPRESSION SUBTRACTIVE HYBRIDISATION AND A CANDIDATE GENE APPROACH.

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Distal Mouse Chromosome (Chr) 2 is subject to genomic imprinting, the phenomenon whereby genes are differentially expressed according toparental origin. Two paternally expressed genes have been identified on distal Chr 2: Gnas, imprinted tissue specifically, and Nnat. The aim is to identify other imprinted genes by suppression subtractive hybridisation and a candidate gene approach. Two separate suppression subtractive hybridisations have been carried out to enrich for paternally and maternally expressed sequences of distal Chr 2. The first used cDNA derived from 15.5 dpc embryos carrying only maternal or paternal copies of distal Chr 2 and the second used cDNA derived from the brains of newborns carrying only maternal or paternal copies of distal Chr 2. Positive controls indicate that subtraction had occurred. Enrichment of the paternally expressed gene Nnat, in the subtractions enhanced for paternally expressed sequences, demonstrated the usefulness of the technique to identify imprinted genes. Both subtracted 15.5 dpc cDNA mixtures have been used as hybridisation probes on cDNA libraries from embryonic material. Three clones showing differential hybridisation are currently being analysed; one is a candidate maternally expressed gene and two are candidate paternally expressed genes. The subtracted newborn cDNA mixtures are currently being used as hybridisation probes on cDNA subtraction libraries.

C. Functional Genomics I/II

Abstracts - Presentations

C1. ESSENTIAL HYPERTENSION - A GENETIC DISEASE DUE TO MANY LITTLE THINGS?

Oliver Smithies. *Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599-7525 USA.*

C2. GENETIC DISSECTION OF ATHEROSCLEROSIS IN MICE.

Nobuyo Maeda. *Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC USA.*

C3. USING KNOCK OUT MICE AND QUANTITATIVE EXPRESSION ANALYSIS TO ADDRESS IMMUNOLOGICAL QUESTIONS.

Catherine Nguyen, *Béatrice Loriod, Geneviève Victorero, Samuel Granjeaud, Bertrand.R. Jordan. TAGC/CIML, Marseille, France.*

C4. HIGHLY EFFICIENT SYNTHESIS OF FULL-LENGTH cDNA BY TREHALOSE THERMO-ACTIVATED REVERSE TRANSCRIPTASE.

Piero Carninci[1], *Masayoshi Itoh[1,2], Yasushi Okazaki[1], Masami Muramatsu[1,2], Yoshihide Hayashizaki[1,2]. [1]Genomic Science Center, Genome Science Lab., RIKEN; [2]CREST JST.*

C5. PROCESSING OF MOUSE GENES ENCODED BY A YAC IN S. CEREVISIAE.

Zdenek Trachtulec, *Jiri Forejt. Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic. Fax No.: *4202 471 3445, e-mail trachtul@biomed.cas.cz.*

C6. THE CHROMOSOME-7 MUTAGENESIS PROGRAM AT THE OAK RIDGE NATIONAL LABORATORY.

E.M. Rinchik, *D.A. Carpenter, E.J. Michaud, D.K. Johnson. Life Sciences Division, Oak Ridge National Laboratory, PO Box 2009, Oak Ridge, Tennessee 37831-8077.*

C7. FUNCTIONAL ANALYSIS OF MAMMALIAN GENES BY A LARGE SCALE GENE TRAP SCREEN IN MOUSE EMBRYONIC STEM CELLS.

Franz Vauti[*], *Michael Wiles[+], Ernst-Martin Füchtbauer[§], Hans-Henning Arnold[#], Harald von Melchner[$], Juergen Otte[$], Carsta Werner[+]; Thomas Metz[*], Wolfgang Wurst[*]. GSF - National Research Center for Environment and Health, Institute of Mammalian Genetics, Ingolstaedter Landstrasse 1, D-85764 Neuherberg; [+]Max-Planck-Institute for Molecular Genetics, Ihnestr. 73, D-14195 Berlin; [§]Max-Planck-Institute of Immunobiology, Department of Developmental Biology, Stübeweg 51, D-79108 Freiburg; [#]Department of Cell- and Molecular Biology, Institute of Biochemistry and Biotechnology, TU Braunschweig, Spielmannstr. 7, D-38106 Braunschweig; [$]Laboratory for Molecular Hematology, University of Frankfurt Medical School, Theodor-Stern Kai 7, D-60590 Frankfurt am Main.*

C8. EXCHANGABLE GENE TRAP AS A TOOL FOR RANDOM MUTAGENESIS.

Kenichi Yamamura, *Yuichi Oike, Takashi Imaizumi, Misao Suzuki, Kimi Araki. Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan.*

C9. THE MOUSE BARE PATCHES AND STRIATED GENE ENCODES A PUTATIVE, NOVEL 3β-HYDROXYSTEROID DEHYDROGENASE.

Gail E. Herman[1], *Xiao Yu Liu[1], Andrew Dangel[1], Wei Zhao[1], Paul Denny[2], Marc Botcherby[3], Bruce Cattanach[2], Jo Peters[2], Ann-Marie Mallon[2], Mark A. Strivens[2], Rachel Bates[2], Webb Miller[4], Michael Rhodes[3], Stephen D.M. Brown[2]. [1]Children's Hospital Research Foundation and Department of Pediatrics, The Ohio State University, Columbus, Ohio 43205, USA. [2]MRC Mouse Genome Centre and Mammalian Genetics Unit, Harwell, OX11 Oxfordshire, UK. [3]MRC Human Genome Mapping Project Resource Centre, Hinxton, Cambridge, UK. [4]Department of Computer Science, The Pennsylvania State University, University Park, Pennsylvania 16802, USA.*

C10. A HIGH RESOLUTION PHYSICAL AND TRANSCRIPT MAP OF THE SATIN - CONGENITAL HYDROCEPHALUS REGION OF MOUSE CHROMOSOME 13.

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C11. GENETIC DISSECTION OF GLUCOCORTICOID RECEPTOR FUNCTION.

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C12. MULTIPLE DEFECTS IN HIPPOCAMPAL FUNCTIONS IN PHOSPHOLIPASE-C-β1 DEFICIENT MICE.

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C13. GENETIC ANALYSIS OF THE MICROPHTHALMIA FAMILY OF bHLHZip TRANSCRIPTION FACTORS.

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C14. ENU INDUCED EMBRYONIC LETHAL MUTATIONS OF THE QUAKING GENE: CONTRASTING EFFECTS.

Roger D. Cox[1], *Alison Hugill*[1], *Alexandra Shedlovsky*[2], *Janice K. Noveroske*[3], *Steve Best*[1], *Monica J. Justice*[3,4], *Hans Lehrach*[5], *William F. Dove*[2]. [1]*Wellcome Trust Centre For Human Genetics, University of Oxford, Windmill Road, Headington, Oxford OX3 7BN, UK.* [2]*McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue Madison, Wisconsin 53706, USA.* [3]*The University of Tennessee, Knoxville, Tennessee 37931.* [4]*Oak Ridge National Laboratory, Y-12 Bear Creek Road Oak Ridge, Tennessee 37831-8080.* [5]*Max Planck Institute for Molecular Genetics,§Ihnestraße 73,D-14195 Berlin (Dahlem), Germany.*

C15. CHOLESTEROL AND THE HEDGEHOG SIGNAL TRARMUCTION PATHWAY: EVIDENCE FOR AN INTERACTION WITH HORNOCYSTEINE METABOLISM AND IMPLICATIONS FOR DEVELOPMENTAL DEFECTS.

Joseph H. Nadeau[1], *Sheila R. Ernest*[1], *Benedicte Christiansen*[2,3], *Brian Gilfix, Orval Mamer, Patrick J. Dunn*[1], *Angela Hosack*[2], *James McGrath*[4], *Allen Bale*[4], *Rudi Balling*[5], *David Sankoff*[6], *David S. Rosenblatt*[2]. [1]*Genetics Department, Case Western Reserve University School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106;* [2]*Departments of Human Genetics, Medicine, Pediatrics, Biology, McGill University, Montreal, Quebec 113A IAI, Canada;* [3]*Present address: Avdeling for medisin sk genetikk, Uffeval sykhus, N-0407 Oslo, Norway;* [4]*Genetics Department, Yale University School of Medicine, 333 Cedar St., New Haven CT 06520;* [5]*Institut hir Saurtiergenetik, GSF Forschungszentrum, Ingolstadter Landstrasse 1, 85758 Neuherberg, Germany;* [6]*Centre de researches mathematiques, Universite de Montreal, Quebec H3C 3J7, Canada.*

C16. *XIST* TRANSGENES ON THE MOUSE Y CHROMOSOME ACT AS A SINGLE COUNTING CENTRE *IN VIVO*.

Miranda Ager[1], *Sarah Duthie*[2], *Susan Morse*[1], *Neil Brockdorff*[2], **Steve Brown**[1]. *1. MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell, UK. 2. X-inactivation Group, MRC Clinical Sciences Centre, London, UK.*

C17. FULL-LENGTH MOUSE cDNA ANALYSIS BY AUTOMATED FLUORESCENT 384 CAPILLARY SEQUENCER SYSTEM (RISA: RIKEN INTEGRATED SEQUENCE ANALYSIS SYSTEM).

Yoshihide Hayashizaki[1,2], *Yasushi Okazaki*[1], *Jun Kawai*[1,2], *Piero Carninci*[1], *Kazuhiro Shibata*[1], *Masayoshi Itoh*[1,2], *Minako Tateno*[1,2], *Nobuya Sasaki*[1,2], *Hideki Konno*[1,2], *Yuichi Sugahara*[1,2], *Shigeru Kawahire*[1], *Masaki Izawa*[1,3], *Yuko Shibata*[1,3], *Masanori Watahiki*[3], *Yuko Yoneda*[3], *Tanaka Tanaka*[4], *Shuji Matsuura*[4], *Massami Muramatsu*[1,2]. [1]*Genomic Science Center, Genome Science Lab., RIKEN;* [2]*CREST JST;* [3]*Nippon Gene Co., Ltd.;* [4]*Wako Pure Chemical Industries, Ltd.*

C18. POSTNATALLY INDUCED INACTIVATION OF gp130 IN MICE RESULTS IN NEUROLOGICAL, CARDIAC, HEMATOPOIETIC, IMMUNOLOGICAL, HEPATIC AND PULMONARY DEFECTS.

Ulrich A.K. Betz[*] *Wilhelm Bloch*[§], *Maries van den Broek*[||], *Kanji Yoshida*[¶], *Tetsuya Taga*[**], *Tadamitsu Kishimoto*[§§], *Klaus Addicks*[§], *Klaus Rajewsky*[*], **Werner Müller**[*]. [*]*Institute for Genetics, University of Cologne, Im Weyertal 121, D-50931 Cologne, Germany;* [§]*Institute for Anatomy I, University of Cologne, Joseph-Stelzmann-Straße 9, D-50931 Cologne, Germany;* [||]*Institute for Experimental Immunology, University Zürich, Switzerland;* [¶]*Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan;* [**]*Department of Molecular Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 101, Japan;* [§§]*Department of Medicine III, Osaka University, Medical School, Osaka 565, Japan.*

C. Functional Genomics I

C1. ESSENTIAL HYPERTENSION - A GENETIC DISEASE DUE TO MANY LITTLE THINGS?

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Humans are a genetically heterogeneous outbred species, but they are nonetheless subject to common diseases having strong genetic components. We are investigating the hypothesis that essential hypertension, one of these diseases, is caused primarily by combinations of quantitative genetic variants that individually have only modest effects. To test this hypothesis we have developed a gene targeting approach which allows the level of expression of chosen genes to be varied systematically in different animals by varying the number of functional copies of the target gene from 1 through 4. We have applied this "gene titration" method to several genes in the renin-angiotensin system and in the natriuretic peptide system. These studies are helping us to understand how complex quantitative traits, such as essential hypertension, are genetically controlled and are demonstrating homeostatic changes that are induced by natural genetic variability.

C2. GENETIC DISSECTION OF ATHEROSCLEROSIS IN MICE.

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Cardiovascular diseases resulting from atherosclerosis account for a large proportion of morbidity and mortality in the United States. The genetic makeup of an individual is clearly important in the etiology of the disease, and molecular approaches based on human population and family studies have successfully identified various factors associated with its incidence. However, the genetic heterogeneity of humans makes it difficult to dissect the roles of individual genetic factors in the development of atherosclerosis and to determine fundamental cause and effect relationships. In addition, environmental factors influence the development of the disease are difficult to control in humans. For these reasons, genetically modified mice, generated via gene targeting in embryonic stem cells or microinjection of DNA into the pronuclei of embryos, have been particularly useful for studying the roles of genetic components in complex genetic diseases. such animals allow us not only to study the phenotypes resulting from specific changes in single genes in vivo, but also to detect synergistic or antagonistic interactions of mutations by combining genetic changes in a single animal.

C3. USING KNOCK OUT MICE AND QUANTITATIVE EXPRESSION ANALYSIS TO ADDRESS IMMUNOLOGICAL QUESTIONS.

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In spite of decades of intensive study, many of the molecular events that occur in the thymus, and that lead to the selection of useful T cells while eliminating unreactive and autoreactive ones, remain poorly known. Gene expression in this complex organ that contains many different cell types is highly specific. Analysis of mouse EST data indicates, for example, that more than half of the sequences found in a thymus cDNA library have no homologue with EST from any other organ. A global view of gene activity in the thymus will certainly help to achieve better understanding of developmental gene regulation and cell to cell interactions in this complex environment.

The vast amount of sequence information becoming available on genes from man and from other species calls for corresponding increases in the rate of collection for data of a more functional nature. Expression measurements often constitute a first step in this direction, and can be performed on a reasonably large scale using highly parallel hybridisation methods (Nguyen et al, 1995; Bernard et al, 1996).

Briefly, a labelled "complex probe" is prepared by reverse transcription and labelling of messenger RNA from the sample (cell type, tissue or surgical sample), and hybridized with an array consisting of many DNA targets, each representing a particular gene. Under the proper conditions, signals measure the relative abundance of each sequence species, and can be acquired quantitatively. The important point is that the combination of a complex probe containing many different mRNA species with a large array of targets allows highly parallel collection of information.

We perform quantitative differential screening by hybridizing complex probes to cDNA libraries gridded on high-density filters and measuring hybridization signals by an imaging plate system. Clones selected on the basis of their expression pattern are then tag-sequenced and further characterized (genome mapping, tissue in situ hybridization...). Our project is developed in an immunology institute, uses a mouse thymus cDNA library, and involves several groups interested in various aspects of thymus development and function. Probes are prepared from various tissues and cell lines, from thymus of mouse knock-out mutants and from embryonic thymus at different gestation times. The same set of clones is screened by all participants, and the data goes back to a common database. Tools for further work (e.g.; a full-length thymus cDNA library, genomic P1 libraries, mapping reagents...) have been acquired and made available. Software tools have been developed to speed data analysis and help correlate it with information contained in public databases.

This highly synergetic approach provides a fairly effective way of interfacing "genomic" approaches with more conventional biological studies.

C4. HIGHLY EFFICIENT SYNTHESIS OF FULL-LENGTH cDNA BY TREHALOSE THERMOACTIVATED REVERSE TRANSCRIPTASE.

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The advent of thermostable enzymes has led to great advances in molecular biology, such as the development of PCR and LCR. However, isolation of naturally thermostable enzymes has been restricted to those existing in thermophylic bacteria. We showed that the disaccharide trehalose enables enzymes to maintain their normal activity (thermoactivation) or even to increase activity (thermoactivation) at high temperatures, at which they are normally inactive (1). We also applied enzyme thermoactivation to improve the reverse transcriptase (RT) reaction. In fact, thermoactivated RT, which displays full activity at 60 °C, is powerful to synthesize full-length cDNA, without early termination usually induced by stable secondary structure of mRNA. This enzyme, which synthesiae very efficiently by the first strand cDNA, together with the improved biotinylated cap trapper technique (2,3), allows the construction of libraries with high content of long full-length cDNAs.

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C5. PROCESSING OF MOUSE GENES ENCODED BY A YAC IN S. CEREVISIAE.

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The processing of mammalian genes encoded within a mouse yeast artificial chromosome (YAC) by the yeast transcription, splicing, and termination apparatus was investigated to evaluate the content of mammalian exons versus "junk" in the YAC clone RNA. All five mouse genes known to be encoded by the YAC were found in the total yeast RNA by reverse transcription (RT) PCR. Controls without RT and treated with Rnase were negative. Of 12 mammalian introns assayed, 6 were correctly spliced by the yeast. Genes were transcribed both from their sense and antisense strands. All microsatellite, inter-repetitive and anonymous mouse loci tested were detected in the YAC RNA. A pair of primers derived from first exons of two head-to-head oriented mouse genes yielded an RT PCR product. An RNA probe, derived from this intergenic region, was protected by the YAC RNA in an Rnase protection assay. These results indicated the presence of false transcription sites in the YAC. Moreover, 3'RACE experiments demonstrated the unability of the yeast to correctly terminate the mouse transcripts. The enrichment of mammalian mRNA versus noncoding sequences in the YAC RNA is low and a method for isolation of mammalian exons based on a YAC clone RNA would produce a high background. Nevertheless, YAC clones can serve as test tubes for investigation of the conservation of gene processing sequences.

C6. THE CHROMOSOME-7 MUTAGENESIS PROGRAM AT THE OAK RIDGE NATIONAL LABORATORY.

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The development of detailed mutation maps of regions of the mouse genome provides new resources for the study of mammalian biology and serves as an important functional complement to the human genome program. Mouse-human linkage homologies permit a type of "surrogate genetics" to be developed for regions of the human genome that is based on analyzing the molecular and organismal consequences of mutations mapping within the corresponding mouse genomic segment. For a number of years, we have been molecularly characterizing regions of mouse chromosome 7 while recovering, in parallel, N-ethyl-N-nitrosourea (ENU)-induced, recessive single-gene mutations mapping within those regions by two-generation hemizygosity screens with radiation-induced deletions. Mutagenesis of one 6- to 11-cM region, surrounding the albino (*c; Tyr*) locus, has been completed, and an on-going screen of an ~4- to 5-cM region, proximal to the pink-eyed dilution (*p*) locus (human 11p and 15q homologies), has so far yielded 19 new mutations, representing 8 complementation groups, from a screen of 1218 gametes. In addition, we shall describe new hemizygosity screens for detecting ENU-induced mutations mapping to the mouse Angelman-Syndrome region, closely linked and distal to the *p* locus, as well as three-generation, homozygosity strategies to induce mutations in proximal Chr 7 (human 19q homology) and mid-Chr 7 (human 15q homology), which are regions that are currently not covered by complexes of deletions. We will also discuss the potential value of "parallel processing" in regional mutagenesis, in which mutations are recovered by three-generation screens with inversions in parallel to (not following) the development of deletions in embryonic stem cells for use as mapping and gene-identification reagents.

[Research sponsored jointly by the Office of Health and Environmental Research, USDOE, under contract DE-AC05-960R22464 with Lockheed Martin Energy Systems, Inc., and by the National Center for Human Genome Research (HG 00370).]

C7. FUNCTIONAL ANALYSIS OF MAMMALIAN GENES BY A LARGE SCALE GENE TRAP SCREEN IN MOUSE EMBRYONIC STEM CELLS.

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We have established a research center in the German HUGO project to perform a large scale functional analysis of mammalian genes taking advantage of the gene trap technology in combination with mouse embryonic stem (ES) cells. The functional analysis of mammalian genes is a fundamental prerequisite for drug discovery and application. The gene trap technology provides an important tool of the human genome project for the identification and characterization of the function of mammalian genes.

We are currently performing a large scale insertional mutagenesis screen in mouse ES cells. The mutagenic events are based on gene trap vector integrations into genes expressed in ES cells, generating a large number of mutant ES cells. The mutated genes are identified using RACE-PCR strategies allowing us to establish an archive of mutated genes generated in ES cells. We will establish mutant mouse lines by germ line transmission if mutated genes relate to human diseases.

At present, we have established 5000 individual, mutated ES cell clones from which 1000 trapped genes have been sequenced (20%). From these data, 390 sequences (39%) show homology to known genes. 210 sequences (21%) have been identified as EST´s and 150 sequences (15%) show no homologies to known sequences present in the NCBI-GenBank database. The individual data for each clone, i.e. sequence, expression pattern and eventually mutant phenotype will be stored in a public database which will be accessible to the scientific community. These data will extend the international EST program and will contribute to unravel gene function genome wide.

C8. EXCHANGABLE GENE TRAP AS A TOOL FOR RANDOM MUTAGENESIS.

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Although the efficient production of mice carrying single germline mutation is possible by use of germline mutagenesis with chemical mutagens or with X-rays, the vast animal housing resource is required and the positional cloning methods are required to identify these mutations. An alternative approach is mutagenesis in embryonic stem (ES) cell. ES cells provide the route of choice for introducing mutated loci into the mouse genome. Since 1989, when the first mice were derived with a locus specifically modified by homologous recombination in ES cells, these techniques have been used to mutate approximately 1% of the total predicted number of mouse loci. However, homologous recombination is laborious and time-consuming; each targeting vector has to be individually constructed and each targeting event must be individually verified. Even with as optimistic estimate of three months of work for generating a targeted ES cell line, comprehensive coverage of the genome would still require some 25,000 person year. Therefore, it is difficult to imagine that the remaining 99% of genes in the mouse can be handled by targeted mutagenesis. A more attracting approach is random insertional mutagenesis in ES cells using gene trap construct. This is an invaluable advantage in an animal with a large genome, small litters and high maintenance costs. We developed a new screening system and a new trap vector. To increase the number of potential target genes in the gene trap experiments, we developed a new screening system in which ES cells are differentiated into embryoid bodies (EB) in suspension culture. We found that the patterns of endoderm gene expression during EB development reflect the order found during mouse development in vivo. One shortcoming of gene trap technology is its relative inability to induce subtle or gain-of-function mutations. To overcome this problem, we developed a new site-specific integration system using the Cre-*lox* recombination system of bacteriophage P1. The *lox* site is composed of an asymmetric 8 bp spacer flanked by 13 bp inverted repeats.

We introduced nucleotide changes in the left 13 bp element (LE mutant *lox* site) or the right 13 bp element (RE mutant lox site). Recombination between the LE mutant *lox* site and RE mutant *lox* site produces a wild-type and a LE+RE mutant site that is poorly recognized by Cre, resulting in stable integration. We applied this system to the gene trap and termed as"exchangable gene trap". Using this system, we can carry out random mutagenesis in the first step, followed by the introduction of any type of mutation or even plasmid to rescue the mouse flanking DNA. Initial studies showed that the targeted genes comprised 50% known gene, 30% expressed sequence tags and 20% novel or unknown genes.

C9. THE MOUSE BARE PATCHES AND STRIATED GENE ENCODES A PUTATIVE, NOVEL 3β-HYDROXYSTEROID DEHYDROGENASE.

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X-linked dominant disorders, which are exclusively lethal prenatally in hemizygous males, have been described in human and mouse. None of these genes has been isolated in either species. We have previously mapped two X-linked dominant male lethal mouse mutations, bare patches (*Bpa*) and striated (*Str*), to an overlapping critical region of 600 kb which is homologous to human Xq283. Based on their clinical phenotype and/or physical location, we and others have proposed that *Bpa* and *Str* may be homologous to human X-linked dominant chondrodysplasia punctata (CDPX2) and incontinentia pigmenti (IP), respectively. Physical contigs in YACs, cosmids, BACs, and PACs have been constructed across the region in human and mouse, and several candidate genes from within the critical region have been isolated in both species. Direct genomic sequencing of all 8 exons of one candidate gene Nsdhl [for NAD(P)H steroid dehydrogenase like] has revealed distinct mutations in 2 independent *Bpa* and 3 independent *Str* alleles. No mutations have been found in 2 additional murine *Bpa* alleles or upon sequencing all 8 exons in human genomic DNA or affected X chromosomal DNA isolated in somatic cell hybrids from 8 independent human CDPX2 females. These results demonstrate that *Bpa* and *Str* are allelic mutations and identify the first mammalian locus associated with an X-linked dominant male lethal phenotype. Genotype/phenotype correlations and possible functions of the gene in intermediary metabolism will be discussed.

C10. A HIGH RESOLUTION PHYSICAL AND TRANSCRIPT MAP OF THE SATIN - CONGENITAL HYDROCEPHALUS REGION OF MOUSE CHROMOSOME 13.

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The proximal region of mouse chromosome 13 harbors numerous interesting mouse mutations that are models for human diseases. For example, the isolation of the putative gene for beige (*Lyst*) has been effective in the identification of the human *CHS*1 gene for Chediak-Higashi syndrome (Jackson, 1997). Recently, a point mutation in *Mf1* has been shown to cause congenital hydrocephalus (*ch*) (Kume et al., 1998). Furthermore, mutations in the human homolog *FKHL7* have been associated with glaucoma and a group of dominant disorders involving changes in the anterior segment of the eye, namely Rieger anomaly, Axenfeld anomaly, and iris hypoplasia (Nishimura et al., 1998). There are four uncloned mutations which map to the proximal region: muted (*mu*), satin (*sa*), crinkled (*cr*), and progressive motor neuropathy (*pmn*) (Stephenson and Lueders, 1997). To understand the molecular basis of these mutations and to investigate their potential for identifying homologous genes that may be involved in disease processes, we have constructed a high resolution genetic and physical map of the proximal region using interspecific crosses anchored at the *ch* locus. In addition, we have isolated several transcripts and are currently investigating them as candidates for these mutations. One of these is an excellent candidate for satin. In parallel, we report the identification of an additional mutant allele of *sa* from an N-ethyl-N-nitrosourea (ENU) mutagenesis screen. Genetic screening utilizing ENU is a powerful method for generating additional mutant alleles which are useful in identification of the causative gene as well as for understanding their function and molecular pathways.

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C11. GENETIC DISSECTION OF GLUCOCORTICOID RECEPTOR FUNCTION.

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Glucocorticoids are involved in numerous physiological processes. Most of the effects are thought to be mediated by the glucocorticoid receptor (GR) via activation and repression of gene expression. Activation requires binding of a receptor dimer while repression is mediated in many cases by protein-protein interaction of GR monomers with other transcription factors.

To analyse receptor function various mutations were generated in the mouse. Mice with a disrupted GR gene (null mutation) die shortly after birth due to respiratory failure indicating an important role of GR in lung function. To separate activating from repressing functions of the GR a point mutation in the D-loop of the receptor, which is required for receptor dimerization, was generated with a Cre-loxP based strategy. Mice carrying this point mutation survive and allow to distinguish between GR functions dependent on DNA binding and those mediated by protein-protein interaction. Since mice with a disrupted GR gene die shortly after birth, cell-specific mutations have been generated with the Cre-loxP system. The GR gene was inactivated in liver, thymus and brain, respectively, to evaluate the role of GR in these organs. Absence of GR in brain leads to alterations in the hypothalamic-pituitary-adrenal axis with increased levels of glucocorticoids and subsequently signs of peripheral hypercorticosteronemia similar to those observed in patients with Cushing's syndrome, namely a growth deficit, osteoporosis and fat tissue redistribution. Interestingly, mutant mice appear to be less anxious revealing an involvement of GR in emotional behaviour. In addition, mutagenesis of GR was restricted to forebrain areas thus allowing the analysis of GR function in higher brain centers.

C12. MULTIPLE DEFECTS IN HIPPOCAMPAL FUNCTIONS IN PHOSPHOLIPASE-C-β1 DEFICIENT MICE.

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Signals from G protein-coupled neurotransmitter receptors are transduced by phosphoinositide-specific phospholipase-C-β (PLCβ) isozymes. We have previously reported the generation and initial characterization of PLCβ1 knock-out mice (Nature 389:290-293). We showed that PLCβ1 was selectively coupled to muscarinic acetylcholine receptor (mAChR) by demonstrating the lack of mAChR signaling in the PLCβ1-/- brain. PLCβ1-/- mice also showed spontaneous epilepsy with accompanying loss of interneurons in the hilus of hippocampus, a similar finding as that in the electrical kindling model of epilepsy. Further analyses have now revealed the followings: 1) PLCβ1 is expressed in hilar interneurons of hippocampus just as mAChR is; its expression is lost in the mutant. The mutant hippocampus shows: 2) sprouting of mossy fibers, axons of the dentate gyrus granule cells, a pathological hallmark in epilepsy, 3) overexpression of BDNF in the granule cells, a finding responsible for the mossy fiber sprouting, and 4) overexpression of neuropeptide-Y, thought to be a compensatory response for seizure. These findings together with previous results led us to conclude that the epilepsy in PLCβ1-/- mice was initiated by dysfunction of the inhibitory interneurons in hippocampus as a result of the defective mAChR/PLCβ1 signaling. This mode of seizure induction contrasts in a mirror image to that in the electrical kindling model, which is initiated by repeated excitatory inputs into hippocampus.

Considering the known cholinergic defect in the brain of Alzheimer's disease, we examined whether the defective mAChR signaling affected the learning ability of PLCβ1-/- mice. We have found that the mutants were severely impaired in hippocampus-dependent learning tasks, including the Morris water maze and the context-dependent fear conditioning. These results indicate that the mAChR/PLCβ1 signaling is necesary for normal function of hippocampus in learning process as well as for controlling the balance between excitation and inhibition in hippocampus. This mutation may be a useful tool for studying the role of cholinergic defect in the pathogenesis of Alzheimer's disease.

C13. GENETIC ANALYSIS OF THE MICROPHTHALMIA FAMILY OF bHLHZip TRANSCRIPTION FACTORS.

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Mutations at the mouse microphthalmia (*Mitf*) locus affect both neural crest- and neuroepithelial derived pigment cells, resulting in loss of coat and eye pigmentation, abnormal eye development (microphthalmia) and hearing loss. Some alleles also result in osteopetrosis and mast cell defects. The many different *Mitf* alleles show distinct modes of inheritance and can be arranged in an allelic series. The *Mitf* gene, a bHLHZip DNA binding protein, can interact in vitro with the closely related transcription factors Tfe3, Tfeb, and Tfec, suggesting that *Mitf* functions as part of a complex network of interacting proteins. To test if such a network exists in vivo, knockout mutations were made in all three *Mitf*-related genes and homozygous phenotypes analyzed as well as phenotypes of different combinations with *Mitf* mutations. Although the embryonic lethality of *Tfeb* homozygotes complicates our analysis, the results show that while Tfec is not involved in *Mitf* function, Tfe3 is clearly important for Mitf in osteoclasts. The severe osteopetrosis observed in homozygotes carrying the semidominant Mitfmicrophthalmia and Mitfmi-OakRidge mutations, can therefore be explained by dominant negative action of those mutant proteins against TFE3 in osteoclasts.

Since mutations in the human *MITF* gene have been shown to be associated with human Tietz syndrome and Waardenburg Syndrome type 2, our studies provide an important mouse model for the disease. Furthermore, the characterization and mutational analysis of *Mitf* and interacting partners serves as a paradigm for the analysis of bHLHZip proteins involved in cancer, such as Myc, Max and Mad. [Research sponsored by the National Cancer Institute under contract with ABL]

C14. ENU INDUCED EMBRYONIC LETHAL MUTATIONS OF THE QUAKING GENE: CONTRASTING EFFECTS.

Roger D. Cox[1], *Alison Hugill*[1], *Alexandra Shedlovsky*[2], *Janice K. Noveroske*[3], *Steve Best*[1], *Monica J. Justice*[3,4], *Hans Lehrach*[5], *William F. Dove*[2]. [1]*Wellcome Trust Centre For Human Genetics, University of Oxford, Windmill Road, Headington, Oxford OX3 7BN, UK.* [2]*McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue Madison, Wisconsin 53706, USA.* [3]*The University of Tennessee, Knoxville, Tennessee 37931.* [4]*Oak Ridge National Laboratory, Y-12 Bear Creek Road Oak Ridge, Tennessee 37831-8080.* [5]*Max Planck Institute for Molecular Genetics,§Ihnestraße 73,D-14195 Berlin (Dahlem), Germany.*

The mouse *quaking* (*qk*) gene has several recessive alleles causing either embryonic lethality at around day 9 in utero, $qk^{lethal\text{-}1}$ ($qk^{l\text{-}1}$), qk^{kt1}, qk^{k2} and qk^{kt3} (Shedlovsky et al. 1988, Justice and Bode 1986, Justice and Bode 1988), or dysmyelination in the CNS and PNS resulting in a quaking phenotype accompanied by seizures in adult mice (qk^{viable} (qk^{v}), (Sidman et al. 1964).

A previous study identified a candidate gene, qkI, that contains an RNA-binding domain, and encodes at least three protein isoforms (Ebersole et al. 1996). To confirm and extend the analysis we have determined the partial genomic structure of qkI. Further, we have examined the exons and splice sites for mutations in the lethal alleles $qk^{l\text{-}1}$, qk^{kt1}, qk^{k2} and qk^{kt3}. We have identified the mutations in $qk^{l\text{-}1}$, qk^{k2} and in qk^{kt3}, and will describe these and their effects. Although homozygotes for each allele die as embryos, their phenotypes as viable compound heterozygotes with qk^{v} differ. Compound heterozygous qk^{v} animals carrying qk^{kt1}, qk^{k2} and qk^{kt3} all exhibit a permanent quaking phenotype similar to qk^{v}/qk^{v} animals, whereas $qk^{v}/qk^{l\text{-}1}$ animals exhibit only a transient quaking phenotype. We provide further evidence that the qkI gene functions both in embryogenesis and myelination. And we provide an explanation for the transient quaking phenotype of compound heterozygotes.

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Shedlovsky, A., T. King, W. Dove (1988). "Saturation germ line mutagenesis of the murine t region including a lethal allele at the quaking locus." Proc. Natl. Acad. sci. USA 85: 180-184.

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C15. CHOLESTEROL AND THE HEDGEHOG SIGNAL TRARMUCTION PATHWAY: EVIDENCE FOR AN INTERACTION WITH HORNOCYSTEINE METABOLISM AND IMPLICATIONS FOR DEVELOPMENTAL DEFECTS.

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Anomalies in folate metabolism causing hyperhomocysteinernia are found in rare inborn errors of metabolism and are important risk factors for more common diseases and disorders such as cardiovascular disease, neural tube defects, seizures, and other birth defects and adult diseases. Dietary folate supplementation prevents many neural tube defects and is suspected to reduce the risk for cardiovascular disease. The genetic basis for hyperhomocysteinernia is known in some cases but not in others. To identify mouse models for experimental studies of the genetic causes and consequences of hyperhomocsyteinemia, a panel of inbred strains and mutant mice were surveyed. Mutations in the *Apc, Apob, Axin, Bmp5, GIB,* and *T* genes, but not the *A, Lp, Mfl, Pax3, Ptch1* or *twist* genes caused elevated homocysteine levels as compared to their wild-type female sibs. An alternative diet with 4.4-fold folate substantially reduced homocysteinelevels in many of these mice. RNA abundance profiles were evaluated to identify points in the folate metabolic pathway that are modulated by these mutant genes. The *Apob, Gli3* and *Ptch1* mutants altered the RNA abundance of *Ahh, Fbp I, Faicar, Gmt* and *Prg* that are involved in folate metabolism, the Cartl homeobox transcription factor whose function is modulated by dietary folate supplementation, and several other genes such as *GIB, Npyl* and *ANPL*. These results demonstrate that mutations in hedgehog signal transduction and lipid transport disrupt folate metabolism and suggest that the resulting hyperhomocysteinernia may contribute to anencephaly, neural tube defects and perhaps other birth defects and adult diseases in these mice.

C16. *XIST* TRANSGENES ON THE MOUSE Y CHROMOSOME ACT AS A SINGLE COUNTING CENTRE *IN VIVO*.

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The X inactivation centre (Xic) is required for the initiation and propagation in cis of X inactivation. Initiation, often referred to as counting, is thought to involve the blocking of a single Xic per diploid chromosome set, marking that X chromosome to remain active. The X inactive specific transcript (*Xist*) gene, necessary for X inactivation, produces a large non-coding stable transcript that is expressed exclusively from the inactive X chromosome. *Xist* RNA accumulates over the inactive X chromosome in *cis* and is thereby thought to induce propagation of X inactivation. Transgenic YAC and cosmid constructs appear to act as a counting centre *in vitro* in differentiating XY ES cells. However, in targeted *Xist* deletions reported to date, XX cells are still able to recognise the presence of two X chromosomes hence counting occurs. Presumably the *cis*-acting counting elements lie in sequence which is outside the targeted deletions but present in the transgenes.

Here we demonstrate that an *Xist* transgene can function as a counting centre *in vivo*. We have analysed *Xist* expression from a mouse line containing 4 copies of a YAC *Xist* transgene inserted at a single locus on the Y chromosome. Accumulation of stable *Xist* RNA from either the X or the Y chromosome can be seen at the time of inactivation. In addition, in preimplantation embryos, *Xist* accumulated signal is seen only on the Y chromosome demonstrating that the ectopic locus is paternally imprinted in the correct manner. This provides the first demonstration that ectopic *Xist* can act as a counting centre *in vivo* and furthermore indicates that multiple *Xist* copies in *cis* compete for available blocking factor as a single counting centre.

C17. FULL-LENGTH MOUSE cDNA ANALYSIS BY AUTOMATED FLUORESCENT 384 CAPILLARY SEQUENCER SYSTEM (RISA: RIKEN INTEGRATED SEQUEN CE ANALYSIS SYSTEM).

Yoshihide Hayashizaki[1,2], *Yasushi Okazaki*[1], *Jun Kawai*[1,2], *Piero Carninci*[1], *Kazuhiro Shibata*[1], *Masayoshi Itoh*[1,2], *Minako Tateno*[1,2], *Nobuya Sasaki*[1,2], *Hideki Konno*[1,2], *Yuichi Sugahara*[1,2], *Shigeru Kawahire*[1], *Masaki Izawa*[1,3], *Yuko Shibata*[1,3], *Masanori Watahiki*[3], *Yuko Yoneda*[3], *Tanaka Tanaka*[4], *Shuji Matsuura*[4], *Massami Muramatsu*[1,2]. [1]*Genomic Science Center, Genome Science Lab., RIKEN;* [2]*CREST JST;* [3]*Nippon Gene Co., Ltd.;* [4]*Wako Pure Chemical Industries, Ltd.*

We have developed a 384 calillary sequencing system (RISA: RIKEN Integrated Sequence Analyzer). This is composed of automated fluorescent 384 capillary sequencers and a plasmid preparator that can extract a total of 40,000 plasmid clones in one day per a machine. A single automated sequencer can now process up to 2304 samples per day. Development of transcriptional sequence will also be demonstrated. 1,2.

Full-length cDNA libraries based on the biotinylated cap trapper method 3,4 have been developed and constructed. These full-length cDNA clones were picked into 384 plates using Q bots (Genetix, Inc.) and stored at -80 C. We are now establishing RISA system and applying it to sequence 5i- and 3i- ends of cDNA. We have already sequenced 7,000 species of mouse full length cDNA and are planning to sequence 1,000,000 runs in a coming year and categorizing the mouse full-length cDNAs.

1. Sasaki et al. PNAS 95, 3455-3460.

2. Izawa et al. JBC273, 14242-14246

3. Carninci et al. Genomics 37, 327-336

C18. POSTNATALLY INDUCED INACTIVATION OF gp130 IN MICE RESULTS IN NEUROLOGICAL, CARDIAC, HEMATOPOIETIC, IMMUNOLOGICAL, HEPATIC AND PULMONARY DEFECTS.

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The pleiotrophic but overlapping functions of the cytokine family that includes interleukin-6 (IL-6), interleukin-11 (IL-11), leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin-1 (CT-1) are mediated by the cytokine receptor subunit gp130 as the common signal transducer. While mice lacking individual members of this family display only mild phenotypes, animals lacking gp130 are not viable. To assess the collective role of this cytokine family, we inducibly inactivated gp130 via Cre-loxP-mediated recombination in vivo. Such conditional mutant mice exhibited neurological, cardiac, hematopoietic, immunological, hepatic and pulmonary defects, demonstrating the widespread importance of gp130-dependent cytokines.

C. Functional Genomics I/II

Abstracts - Posters

C19. UNUSUAL GENETIC PROPERTIES OF MICE HETEROZYGOUS AT THE *Om* LOCUS.

Patricia Baldacci, *Stéphanie Le Bras, Michel Cohen-Tannoudji, Chantal Kress, Sandrine Vandormael, Charles Babinet. Biologie du Développement, Dept. d'Immunologie, Institut Pasteur, 25, Rue du Dr Roux, 75724 Paris Cedex 15 France. Tel: 33-1-45-68-84-86, Fax: 33-1-45-68-86-34, email:baldacci@pasteur.fr*

C20. REGULATION OF MOUSE HEPATOCARCINOGENESIS BY GROWTH HORMONE.

James Bugni, *Therese Poole, Norman Drinkwater. McArdle Laboratory University of Wisconsin, Madison, WI 53706. bugni@oncology.wisc.edu*

C21. IDENTIFICATION OF GENES THAT COOPERATE WITH *TPL2*.

Jeffrey D. Ceci[1], *Jonathan Shafer*[1], *Nancy A. Jenkins*[2], *Neal G. Copeland*[2], *Philip N. Tsichlis*[3]. [1]*Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77555-0643,* [2]*NCI-FCRDC, Frederick, MD 21702,* [3]*Department of Microbiology/Immunology, Thomas Jefferson University, Philadelphia, PA.*

C22. GASTRIN DEFICIENT MICE HAVE REDUCED ANTRAL EXPRESSION OF IAPP AND PYY.

L. Friis-Hansen, *J.F. Rehfeld, L.C. Samuelson, F. Sundler. Dept. of Clinical Biochemistry, University of Copenhagen, Denmark; Dept. of Physiology, University of Michigan, Ann Arbor, MI, USA; Dept. of Physiology and Neuroscience, University of Lund, Sweden.*

C23. CHARACTERIZATION OF Xpg-DEFICIENT MICE.

Yoshi-nobu Harada, *Naoko Shiomi, Tadahiro Shiomi.*

C24. ANALYSIS OF THE EFFECT OF THE ACUTE MYELOID LEUKAEMIA ASSOCIATED t(8:21) AND inv(16) HUMAN FUSION GENES ON DEFINITIVE HAEMATOPOIESIS IN TRANSGENIC MICE.

Soren Warming, Jesper Laursen, **J. Peter Hjorth.** *Department of Molecular and Structural Biology, University of Aarhus, C.F. Mollers Alle, Building 130, DK-8000 Arhus, Denmark.*

C25. TRANSCRIPTION AND SPLICING OF MAMMALIAN GENES IN *Saccharomyces cerevisiae*: A NEW APPROACH FOR EXON-TRAPPING?

Bärbel Kunze[1], *Thomas Hellwig-Bürgel*[2], *Dieter Weichenhan*[1], *Walther Traut*[1]. [1]*Institut für Biologie,* [2]*Institut für Physiologie Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-23538 Lübeck.*

C26. GENOMIC ORGANIZATION, EXPRESSION PATTERN AND TARGETING OF Pkd2, THE MURINE HOMOLOGUE TO HUMAN PKD2.

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C27. PHENOTYPE- AND GENE-BASED MUTAGENESIS SCREENS IN MICE AND ES CELLS.

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C19. UNUSUAL GENETIC PROPERTIES OF MICE HETEROZYGOUS AT THE *Om* LOCUS.

Patricia Baldacci, *Stéphanie Le Bras, Michel Cohen-Tannoudji, Chantal Kress, Sandrine Vandormael, Charles Babinet. Biologie du Développement, Dept. d'Immunologie, Institut Pasteur, 25, Rue du Dr Roux, 75724 Paris Cedex 15 France. Tel: 33-1-45-68-84-86, Fax: 33-1-45-68-86-34, email:baldacci@pasteur.fr*

The *Om* locus, described in the DDK strain of mice, affects preimplantation embryonic development. Embryos from a cross between DDK females x non-DDK males die at the morula to blastocyst stage (referred to as the DDK syndrome) whereas the reciprocal cross, non-DDK females x DDK males, produces viable offspring. The death of F1 embryos is due to a defective interaction between a maternal DDK cytoplasmic factor and the paternal non-DDK pronucleus. The locus *Om* has been mapped to chromosome 11, close to the *Scya* family of genes. We have established three congenic lines carrying the *Om* DDK alleles on a BALB/c background. We will present data showing that heterozygous mice of these strains have some very unusual genetic properties:

1) the heterozygous females have a DDK phenotype. This result is contrary to the predictions made by Wakasugi in his model for *Om*. We propose that this unusual phenotype is due to a differentiel expression or allelic exclusion in favour of the *Om* DDK allele during oogenesis;

2) the heterozygous males transmit the non-DDK allele to a significant proportion of offspring, indicating that the DDK allele can attenuate the non-DDK allele during spermatogenesis. This could occur via paramutagenic interactions, as described in plants and Drosophila and more recently for susceptibility to diabetes, or alternatively at the level of protein interactions;

3) finally, depending on the parental genotypes around *Om*, a maternal transmission ratio distorsion can be observed.

C20. REGULATION OF MOUSE HEPATOCARCINOGENESIS BY GROWTH HORMONE.

James Bugni, *Therese Poole, Norman Drinkwater. McArdle Laboratory University of Wisconsin, Madison, WI 53706. bugni@oncology.wisc.edu*

Male mice are significantly more susceptible to liver tumor development than female mice. To test the hypothesis that growth hormone mediates the sex-dependent regulation of hepatocarcinogenesis we are using growth hormone-deficient C57BL/6J-*lit/lit* (*little*) mice. We have found that in both males and females, *little* mutant mice had significantly fewer liver tumors than wild type C57BL/6J mice following induction with the carcinogen N,N-diethylnitrosamine (DEN) (p-values $< 10^{-6}$). Wild type males had 57-fold more tumors than *little* males at 32 weeks of age and 36-fold more at 50 weeks. Wild type females had 11-fold more tumors than *little* females at 50 weeks. Furthermore, both wild type and *little* mutant mice activate DEN with the same activity, but *little* mutants develop preneoplastic foci at a much slower rate than wild type mice. These results suggest that wild type levels of growth hormone act as a tumor promoter and do not affect the rate of initiation. We are currently examining whether the sex differences in susceptibility are dependent on growth hormone. In order to identify liver specific mediators of tumor promotion we are using the technique of Representational Difference Analysis (RDA) to identify growth hormone-regulated genes in the liver. In screens comparing the livers of wild type to *little* mice we identified 12 genes that are either up-regulated or down-regulated by growth hormone. Differential expression in some cases has been previously reported, and in the other cases, it was confirmed by either Northern Blotting or RT-PCR. Three of the genes identified are novel.

C21. IDENTIFICATION OF GENES THAT COOPERATE WITH *TPL2*.

Jeffrey D. Ceci[1], *Jonathan Shafer*[1], *Nancy A. Jenkins*[2], *Neal G. Copeland*[2], *Philip N. Tsichlis*[3]. [1]*Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77555-0643,* [2]*NCI-FCRDC, Frederick, MD 21702,* [3]*Department of Microbiology/Immunology, Thomas Jefferson University, Philadelphia, PA.*

Previous studies have shown that proviral insertion into the last intron of tumor progression locus-2 (*Tpl2*) in retrovirally-induced rat T-cell lymphomas results in the enhanced expression of a C-terminally truncated TPL2 kinase. *In vitro* studies have shown that the truncated protein exhibits a seven-fold higher catalytic activity and is three-fold more efficient than the wild-type protein in activating the Map kinase (MAPK) and stress-activated protein kinase (SAPK) signal transduction pathways. We have generated transgenic mice expressing either wild type or truncated protein to determine whether overexpression has an effect *in vivo*. Transgenic mice expressing two forms of the truncated cDNA developed either T-cell lymphoblastic lymphomas or thymomas. The time course of tumor development in these mice suggests that other proto-oncogenes or tumor suppressor genes cooperate with *Tpl2* in the progression of these tumors. The main objective of this project is to identify and characterize such genes. To address these goals, we have infected these two transgenic mouse lines with Moloney murine leukemia virus (MoMuLV) to accelerate the time course of tumorigenesis, and to facilitate the identification and cloning of cooperating genes. MoMuLV infection of one of the transgenic lines resulted in an accelerated rate of lymphoblastic lymphoma tumor development, as we predicted (the other line is currently being analyzed). We have started to identify tumor-promoting common sites of viral integration into the DNA of these tumors, and will then clone these sites to identify and characterize candidate genes that may cooperate with *Tpl2* during tumorigenesis. We will be presenting our initial results. These studies should provide valuable insights into the sequence of genetic events required for the progression of *Tpl2*-induced T-cell tumorigenesis, and potentially of other T-cell induced tumors.

C22. GASTRIN DEFICIENT MICE HAVE REDUCED ANTRAL EXPRESSION OF IAPP AND PYY.

L. Friis-Hansen, *J.F. Rehfeld, L.C. Samuelson, F. Sundler. Dept. of Clinical Biochemistry, University of Copenhagen, Denmark; Dept. of Physiology, University of Michigan, Ann Arbor, MI, USA; Dept. of Physiology and Neuroscience, University of Lund, Sweden.*

Hormones secreted from the antrum play an important role in the regulation of gastric physiology, most important are gastrin and somatostatin. Two of the newer hormones identified in the antrum are IAPP and PYY, which have been implicated in modulation of gastric acid secretion and protection against ulcers. Using the gastrin deficient mouse as a model, we wanted to study how lack of gastrin and lowered gastric acidity affects the expression of these hormones.

The number of somatostatin and PYY immunoreactive (IR) cells was reduced by 50% in the gastrin deficient mice. Furthermore, semi-quantitative in-situ for somatostatin showed a decrease in somatostatin mRNA. In the gastrin deficient mice, there was no IAPP IR cells. Northern blotting and quantitative RT-PCR confirmed the reduction in PYY and IAPP expression. PYY IR cells often co-expressed IAPP and both hormones were often found co-localized in somatostatin IR cells in the antrum. There was little co-localization between gastrin and PYY or IAPP. Re-infusion of gastrin into gastrin deficient mice for 1 week did not change the expression pattern of PYY and IAPP and did not cause a normalization of conditions in the antrum.

We conclude that expression of IAPP and PYY is reduced in gastrin deficient mice and it is not influenced by plasma gastrin conc. Instead it seens likely that expression of IAPP and PYY, like somatostatin, is influenced by changes in gastric acidity.

C23. CHARACTERIZATION OF Xpg-DEFICIENT MICE.

Yoshi-nobu Harada, *Naoko Shiomi, Tadahiro Shiomi.*

Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are two rare inheritable disorders, which are characterized by a clinical and cellular hypersensitivity to the ultraviolet (UV) component of the sunlight spectrum. XP-G is one of the most rare and phenotypically heterogeneous groups of XPs. In a few cases, a combination of clinical hallmarks of XP-G and CS has been reported. The human XPG gene has been shown to have a function of structure specific endonuclease. However, this function deduced from in vitro experiments is unable to explain the complexed chrinical phenotypes associated with XPG. To investigate further unknown functions of XPG, we have generated two strains of gene disrupted XPG model mice, XPG/2 and XPG/3.

XPG/2 and XPG/3 has been established by disrupting the third and the last fifteenth exon of the mouse Xpg gene, respectively. The defective genes of mouse Xpg allele have been transmitted in Mendelian manner. In XPG/2 strain, growth of the mutant homozygotes was severely inhibited and they died before weaning. The fibroblast cells from XPG/2 mutant homozygotes were hypersensitive to UV (254 nm) irradiation as well the cells from severe XP-G patients. On the other hand, mutant homozygotes of XPG/3 mice have grown normal and were fully fertile. The fibroblast cells from XPG/3 mutant homozygotes were also hypersensitive to UV irradiation, but the sensitivity of the cells was intermediate between wild type and XPG/2 mutant homozygotes.

Anatomical observations for the XPG/2 mutant homozygotes suggested that they were in a state of starvation atrophy. Morphological analysis showed that new born mice of the XPG/2 had immature villi of small intestines. Because the severe runting and premature death of the XPG/2 mice is similar to those of CS patients, the XPG/2 mice must be a good model for XP-G patients combined with CS.

C24. ANALYSIS OF THE EFFECT OF THE ACUTE MYELOID LEUKAEMIA ASSOCIATED t(8:21) AND inv(16) HUMAN FUSION GENES ON DEFINITIVE HAEMATOPOIESIS IN TRANSGENIC MICE.

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The evolutionary conserved heterodimeric transcription factor CBF consists of two subunits AML1 and CBFb. Both *AML1* and *CBFB* are involved in human acute myeloid leukaemia (AML). Translocation (8;21)(q22;q22) is highly associated with a subtype of AML that shows granulocytic maturation (FAB M2) and has been shown to generate a fusion gene , *AML1-ETO*, consisting of sequences derived from *AML1* and the gene coding for the *ETO* transcription factor. Inversion (16)(p13;q22) is a marker of a subtype of AML with eosinophilic blasts (FAB M4Eo) and this chromosomal abnormality contains another fusion gene, CBFB-MYH11, consisting of the 5′ part of *CBFB* and the coding sequences for the tail domain of smooth muscle myosin heavy chain. Knock-in models have shown that both the AML1-ETO and CBFβ-MYH11 fusion genes inhibit murine fetal haematopoiesis and cause embryonic lethality in heterozygous mice. to circumvent the embryonic lethality of these human fusion genes we have derived transgenic mice carrying the coding sequences for AML1-ETO or CBFβ-MYH11 under control of the myeloid specific hCD11b promoter or the promoter from the promyelocytic specific hCathepsin G gene. Expressing lines were selected by RT-PCR analysis of bone marrow RNA. A low number of transgenic mices aged between 9 and 15 months have been analyzed by flow cytometry using monoclonal antibodies against myeloid markers Mac-1 and Gr-1. In rare cases, bone marrow abnormalities were observed. Most strikingly, there were two cases where the number of Mac-1^{pos} and Gr-1^{low} cells was increased, suggesting that a population of relatively undifferentiated myeloid cells had expanded. We are currently establishing myeloid specific retroviral tagging systems in lines from all four types of transgenic mice to identify genes that cooperate with *AML1-ETO* or *CBFB-MYH11* in the develop-

C25. TRANSCRIPTION AND SPLICING OF MAMMALIAN GENES IN *Saccharomyces cerevisiae*: A NEW APPROACH FOR EXON-TRAPPING?

Bärbel Kunze[1], *Thomas Hellwig-Bürgel*[2], *Dieter Weichenhan*[1], *Walther Traut*[1]. [1]*Institut für Biologie,* [2]*Institut für Physiologie Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-23538 Lübeck.*

Members of the murine *Sp100-rs* gene family are composed of several exons and introns, the latter ones of considerable length (up to 20 kb). This gene-family encodes a transcript family. In C57BL/6 a predominant 3.0 kb transcript showed up in Northern hybridizations, additional ones were detected by RT-PCR. We isolated a yeast artifical chromosome (YAC) clone (yMm75) from the Princeton YAC-library which contains three different copies of the murine *Sp100-rs* gene family. In total RNA from this YAC-clone we detected a 3.0 kb *Sp100-rs* transcript as well. This indicates transcription of the mouse-derived YAC-insert. In case of promoters with canonical TATA-boxes the transcription machinery is well conserved from yeast to mammals (1). The *Sp100-rs* promoter region does not contain typical TATA or CCAAT boxes. However, the promoter shows an initiator (Inr) element, a potential Sp1 binding site and, close to this a potential housekeeping initiator protein 1 (HIP1) binding site. Cloning and sequencing of *Sp100-rs* specific RT-PCR products showed, that the exons were precisely spliced in yeast. This capability is unexpected because gene-organization in yeast differs significantly from that in metazoa. Only 2-5% of the known nuclear yeast genes contain an intron, only few of them are larger than 400 bp (2). Furthermore, the use of consensus splice sites is more strict in yeast than in mammals (3). We sequenced two of the *Sp100-rs* introns (2.5 kb and 0.6 kb), which were precisely spliced out in *S. cerevisiae*. Inverted repeats found in the sequences may form secondary structures which enable the yeast transcription machinery to splice out these large mammalian introns. Such a mechanism was postulated for the few large yeast introns (3). If transcription and splicing of mammalian genes is a general property of yeast, this system could offer an alternative method to conventional exon-trapping systems in tissue culture cells.

(1) Cavallini B et al. (1988) Nature, 334, pp77-80; (2) Rymond BC and Rosbash M (1992), in The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression , Vol II, pp 143-192, Cold Spring Harbor Laboratory Press; (3) Parker R and Patterson B (1987), in Molecular Biology of RNA, pp133-149, Academic Press.

C26. GENOMIC ORGANIZATION, EXPRESSION PATTERN AND TARGETING OF Pkd2, THE MURINE HOMOLOGUE TO HUMAN PKD2.

Petra Pennekamp[1], *Wilda M*[2], *Bogdanova N*[1], *Markoff A*[1], *Hameister H*[2], *Horst J*[1], *Dworniczak, B*[1]. [1]*Institut für Humangenetik, Muenster, Germany;* [2]*Abteilung medizinische Genetik der Universität Ulm, Germany.*

Human polycystic kidney diseases (PKD) form a family of closely related renal disorders which genetically can be divided into two major groups: the autosomal recessive (ARPKD) and the autosomal dominant (ADPKD) forms. ADPKD is one of the most commonly inherited diseases with an attributed frequency of at least 1:1000 in Caucasians (Dalgaard, 1957). It is generally characterized by progressive development and enlargement of multiple fluid filled cysts in the kidneys that frequently result in chronic and end-stage renal failure. ADPKD accounts for about 8-12% of all patients requiring haemodialysis world-wide. So far two genes, *PKD1* and *PKD2* have been identified as causative determinants for the disease and there is convincing evidence that at least a third *ADPKD* locus, yet to be mapped, is also involved.

In order to study the disease causing mechanisms for ADPKD in more detail and the interactions of the responsible gene products in vivo it is evidently beneficial to develop animal models which are insufficient in one or both gene functions.

In order to create a mouse model for the second form of ADPKD, *Pkd2*, we characterized the mouse *Pkd2* gene and analyzed its expression pattern compared to *Pkd1* during development. FISH and interspecific backcross analysis located *Pkd2* on mouse chromosome 5 proximal to *D5Mit175*. The gene spans at least 35 kb of the mouse genome and contains 15 exons. Its translation product consists of 967 amino acids and the peptide shows a 95% homology to human polycystin2. Functional domains are particularly well conserved in the mouse homologue. In situ hybridization on mouse embryo sections using *Pkd1*- and *Pkd2*-cDNA probes showed mainly coexpression of both polycystins in all stages analysed so far.

Gene targeting of the murine *Pkd2* gene which will lead to the development of a conditional mouse mutant is currently underway. One of the used constructs introduces the LacZ gene in frame into the *Pkd2*-startcodon. From 376 clones which were picked after double selection with G418 and FIAU, 51 have been already proved to be homologous recombined by Southern blot analysis. However, the previously performed PCR screen with two different primer pairs leads us to the hypothesis, that approximately 30-40% of these 376 clones could harbor a targeted allele. Blastocyst injection is ongoing.

C27. PHENOTYPE- AND GENE-BASED MUTAGENESIS SCREENS IN MICE AND ES CELLS.

Lawriston Wilson, Rob Munroe, Maja Bucan, Kerry Schimenti, Doug Pittman,* **John Schimenti.** *The Jackson Laboratory, Bar Harbor, ME USA*; The University of Pennsylvania, Philadelphia, PA USA.*

We have been developing a set of approaches for efficiently creating and identifying mutations in mice. One technology involves generating deletion complexes by irradiation of ES cells. We have generated a series of deletions on Chromosomes 5 and 17 using this approach, and are saturating a region of Chr 5 with ENU-induced recessive mutations that are uncovered by the deletions. To facilitate the process of generating deletions, we are constructing "Delbank," a collection of ES cell clones bearing mapped insertions of a TKneo cassette. These clones serve as the starting point for inducing deletions virtually anywhere in the genome with a minimum of effort. Another strategy we are developing that enables both gene-based and phenotype-based mutagenesis involves chemical mutagenesis of ES cells. We have defined conditions of chemical mutagenesis whereby treated cultures demonstrate a marked increase in mutations at the *HPRT* locus yet retain the ability to undergo germline transmission. Variations of this general approach are being used to create mice for phenotype screening, perform phenotype screens directly in cells, and to establish a bank of mutagenized clones that can be molecularly screened for point mutations.

D. Quantitative and Mutagenic Traits

Abstracts - Presentations

D1. MAPPING OF QTLS FOR BODY WEIGHT AND FATNESS FROM A HIGH GROWTH SELECTED MOUSE LINE.

G.A. Brockmann*, *C.S. Haley†, S. Karle, S.A. Knott#, U. Renne*, M. Schwerin*. *Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany; †Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom; #Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom.*

D2. COLON CANCER SUSCEPTIBILITY GENES.

Tom van Wezel, *Claudia Ruivenkamp, Fons Stassen, Peter Demant. The Netherlands Cancer Institute, Department of Molecular Genetics, Plesmanlaan 121 1066 CX Amsterdam.*

D3. MAPPING DISEASE MODIFIER LOCI FOR MALARIA AND LEISHMANIA.

Simon Foote, *Lynden Roberts, Rachel Burt, Vikki Marshall, Tracey Baldwin, Emanuela Handman, Andrew Lew, Andrew Roberts. The Walter and Eliza Hall Insitute of Medical Research, P.O. Royal Melbourne Hospital, Parkville 3050, Australia.*

D4. MARKER-ASSISTED CONGENIC STRATEGY IN COMBINATION WITH HISTORICAL RECOMBINANTS FOR PINPOINTING CANDIDATE GENES FOR COMPLEX TRAITS: APPLICATION FOR AN INSULIN-DEPENDENT DIABETES SUSCEPTIBILITY GENE (*Idd3*) OF THE NONOBESE DIABETIC (NOD) MOUSE.

Hiroshi Ikegami, *Susumu Makino, Yoshihiko Kawaguchi, Toshio Ogihara. Department of Geriatric Medicine, Osaka University Medical School, Osaka, *Shionogi and Co. Ltd., Shiga, Japan.*

D5. FROM QUANTITATIVE TRAIT LOCUS TO GENE: THE CLONING OF *Ath1* AND *Lith1*.

Beverly Paigen, *David Beier, Shelley Phelan, Frank Lammert, David Wang, Martin Carey. The Jackson Laboratory, Bar Harbor, ME 04609 and Brigham and Women's Hospital, Boston MA 02115.*

D6. THE GENETIC DISSECTION OF LOW IgE RESPONSE IN THE SJL/J MOUSE INBRED STRAIN.

John Smutko, Jim Vitale, Paul Markel, Bill Paul, Tomo Yoshimoto*, Cyndy Watson*,* **Karen J. Moore**. *Genetic Systems, Millennium Pharmaceuticals Inc., 640 Memorial Drive, Cambridge MA 02139. *Laboratory of Immunology, NIAID, NIH, Bethesda, MD.*

D. Quantitative and Mutagenic Traits

D1. MAPPING OF QTLS FOR BODY WEIGHT AND FATNESS FROM A HIGH GROWTH SELECTED MOUSE LINE.

G.A. Brockmann*, *C.S. Haley†, S. Karle, S.A. Knott#, U. Renne*, M. Schwerin**. **Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany; †Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom; #Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom.*

Quantitative trait loci (QTLs) influencing body weight were mapped by linkage analysis in crosses between a high body weight selected line (DU6) and a control line (DUKs). The two mouse lines, which were generated from the same base population and maintained as outbred colonies, differ in mean body weight by 106% and in mean abdominal fat weight by 100% at 42 days. A QTL analysis was performed using structured F_2 pedigrees derived from crosses of a single male from DU6 with a female from DUKs. QTLs significant at the genome wide level were mapped for body weight on chromosome *11*, for abdominal fat weight on chromosomes *4*, *11*, and *13*, and for abdominal fat percentage on chromosomes *3* and *4*. The strong effect on body weight of the QTL on chromosome *11* was confirmed in three independent pedigrees. The effect was additive and independent of sex, accounting for 21 to 35% of the phenotypic variance of body weight within the corresponding F_2 populations. The test for multiple QTLs on chromosome *11* with combined data from all pedigrees indicated the segregation of two loci separated by 36 cM influencing body weight.

The results were verified in two additional different crosses: (a) DU6 (high growth selected line) x DBA/2 (inbred line, low body weight), and (b) NMRI (high growth selected line) x DBA/2. These analyses support the conclusion that the identified chromosomal regions harbour genes which account for elevated body weight and fat accumulation.

Mice from the F_2 population of the cross between DU6 and DBA/2 were chosen for the production of congenic strains, in which regions of the chromosomes 11 and 13 of line DU6 are being transferred to DBA background. After repeated back-crossing the chromosomal regions for the most likely QTL positions have been confirmed.

The expression of candidate genes has been studied by parallel hybridization of entire cDNA populations of pooled samples from DU6 and DUKs animals to Atlas cDNA Expression Arrays, which harbor hundreds of genes which have been assigned to functional classes and which are under tight transcriptional control. The comparison of gene expression patterns in different tissues give a hint on genes which might be involved in selection for high body weight in line DU6.

D2. COLON CANCER SUSCEPTIBILITY GENES.

Tom van Wezel, *Claudia Ruivenkamp, Fons Stassen, Peter Demant. The Netherlands Cancer Institute, Department of Molecular Genetics, Plesmanlaan 121 1066 CX Amsterdam.*

Colorectal carcinogenesis is a complex multistep process controlled by different sets of genes. We analyzed the genetic control of colon cancer susceptibility in the mouse. To identify susceptibility genes involved in the genetic predisposition to sporadic colon cancer we use the Recombinant Congenic Strains (RCS). We have mapped nine loci that control the susceptibility to colorectal cancer; *Scc1* and *Scc2* on chromosome 2 and *Scc3*, *Scc4* and *Scc5* on chromosomes 1, 17 and 18 respectively (van Wezel et.al., Nature Genetics 1996). Additional loci have been mapped to chromosomes 3, 8, 10 and 11. Most and probably all of these loci are different from the known oncogenes and tumorsupressor. Interestingly, several of these loci show mutual interactions: the individual alleles of these loci are not intrinsically susceptible or resistant, but their effect is influenced by the genotype at the interacting locus. We have also found overlap between regions involved in cancer susceptibility in different tissues. Currently we are studying these regions in more detail to establish whether these effects are due to a single gene or to clusters of susceptibility genes.

The *Scc1* locus on chromosome 2 has been studied closely in order to identify candidate genes. Mice with a recombination in the *Scc1* region have been progeny-tested for susceptibility to DMH induced colon tumors. This resulted in the mapping of *Scc1* to approximately 0.3cM between *D2Nki341* and *D2Mit253*. The availability of 11 recombinants in this region will further increase the precision of the mapping. A contig covering the *Scc1* region has been constructed from different mouse libraries and is being used to build a physical and a transcriptional map.

We are also testing the susceptibility of Scc1 alleles of different, unrelated, mouse strains. Depending on the nature of the candidate genes and the differences between their resistant and susceptible alleles a transgenic or knock-out strategy will be used to confirm their role in susceptibility.

D3. MAPPING DISEASE MODIFIER LOCI FOR MALARIA AND LEISHMANIA.

Simon Foote, *Lynden Roberts, Rachel Burt, Vikki Marshall, Tracey Baldwin, Emanuela Handman, Andrew Lew, Andrew Roberts. The Walter and Eliza Hall Insitute of Medical Research, P.O. Royal Melbourne Hospital, Parkville 3050, Australia.*

The course of infection of either the murine malarial parasite *P. chabaudi* or the kinetoplastid *L. major* differs between inbred strains of mice. Phenotypes were measured in the F2 progeny of large F1 intercrosses and mice were genotyped across the entire genome using microsatellite markers. Two crosses were carried out looking for modifier loci for *P. chabaudi*. C7BL/6 mice were crossed to either C3H/He or SJL mice. These latter two strains are susceptible to infection and die when challenged with an intravenous dose of 104 parasites. 10-15% of all F2 mice succumbed to infection by *P. chabaudi*. Loci controlling outcome to infection mapped to chromosomes 8 and 9 in the the C3H/He x C57BL/6 cross and only to chromosome 9 in the SJL cross. Parasitaemias were measured in all animals on each day of the experiment. A quantitative trait linkage analysis was performed using the Mapmaker Package and the loci mediating disease outcome were also involved in control in the level of parasites. Fine mapping studies of the both loci have been carried out and the critical intervals have been refined to several cM. The level of parasitaemia post crisis in the C3H/He cross follows a normal distribution, allowing the mapping of loci involved in the control of parasitaemia after the specific immune response begins to clear parasites. A locus has been mapped that controls parasite levels after crisis. This is independent of either the chromosome 8 or 9 loci, as these have no effect at this point in the infection.

D4. MARKER-ASSISTED CONGENIC STRATEGY IN COMBINATION WITH HISTORICAL RECOMBINANTS FOR PINPOINTING CANDIDATE GENES FOR COMPLEX TRAITS: APPLICATION FOR AN INSULIN-DEPENDENT DIABETES SUSCEPTIBILITY GENE (*Idd3*) OF THE NONOBESE DIABETIC (NOD) MOUSE.

Hiroshi Ikegami, **Susumu Makino, Yoshihiko Kawaguchi, Toshio Ogihara. Department of Geriatric Medicine, Osaka University Medical School, Osaka, *Shionogi and Co. Ltd., Shiga, Japan.*

Identification of disease-causing mutations of complex traits is a formidable challenge because the effect of each mutation on overall phenotype is subtle and is detectable only in the presence of other background genes. In usual congenic strategy, the presence of a susceptibility gene is indicated when disease protection is observed by introgression of a chromosomal segment from a disease-resistant strain, but disease-causing mutations cannot be pinpointed by this approach. We took different strategy in which a chromosomal segment with the same candidate mutation as, but different flanking markers from, a parental strain is introgressed onto genetic background of the parental strain so that contribution of the candidate mutation to disease development is directly tested. We have applied this approach to a susceptibility gene for insulin-dependent diabetes mellitus (*Idd3*) of the NOD mouse. *Idd3* was previously mapped to proximal chromosome 3 by usual congenic strategy, and a candidate disease gene, *Il2*, encoding interleukin 2, was reported to be allelically variant between NOD and control strains. We searched for recombinant haplotypes (historical recombinants) with the same *Il2* sequence as, but different flanking markers from, the NOD mouse in sister strains derived from the same outbred colony as the NOD mouse. A recombinant haplotype from one of such strains, inbred ICR Shionogi (IIS), has been introgressed onto NOD by marker-assisted congenic strategy, and a congenic NOD.IIS-chr 3 has been established by N5 generation. NOD.IIS-chr 3 mice developed type 1 diabetes regardless of genotypes of the Il2 region, indicating that Il2 from IIS strain confers susceptibility to type 1 diabetes. These data not only provides further evidence that *Il2* is responsible for *Idd3* effect, but also suggest a novel strategy for pinpointing candidate mutations for complex traits.

D5. FROM QUANTITATIVE TRAIT LOCUS TO GENE: THE CLONING OF *Ath1* AND *Lith1*.

Beverly Paigen, *David Beier, Shelley Phelan, Frank Lammert, David Wang, Martin Carey. The Jackson Laboratory, Bar Harbor, ME 04609 and Brigham and Women's Hospital, Boston MA 02115.*

Ath1 was first described as a QTL that affects susceptibility to atherosclerosis and plasma high density lipoprotein cholesterol with lesion size as the measured quantitative trait. The region on Chr 1 containing *Ath1* was narrowed by first constructing congenic strains with susceptible C57BL/6J as background strain and the resistant region of *Ath1* from SPRET/Ei or PERA/Ei. The congenic was backcrossed to C57BL/6J and recombinants in the area phenotyped. As the region was narrowed more, the *Ath1* allele carried by recombinants was determined by testing progeny of the recombinants rather than the recombinants themselves. A 1763-mouse backcross was used to narrow the region to 0.05 cM. A novel antioxidant protein, (*Aop2*), was tested as candidate gene and found to have a sequence difference in the coding region of some resistant strains and an expression difference in others, suggesting that two distinct resistant alleles exist.

Lith1 was first described as a QTL that affects susceptibility to cholesterol gallstone formation with weight of gallstones as the quantitative trait. The region on Chr 2 containing *Lith1* was narrowed by first constructing a congenic strain with the resistant strain AKR/J as the background and the susceptible alleles from C57L/J in the *Lith1* region. This congenic was backcrossed to AKR/J. The region was narrowed first by selective phenotyping of recombinants, and then by progeny testing of recombinants. A 423-mouse backcross was used to narrow the region to 0.2 cM. A novel canalicular membrane transporter, sister to p-glycoprotein (*spgp*), was tested as candidate gene and found to have an expression difference in mRNA and protein concentrations.

Several lessons were learned from the cloning of these QTLs. First, starting with a large difference that accounted for a large fraction of the variance was critical. Second, the construction of a congenic that isolated one QTL from other genes affecting the trait is very helpful. Third, a series of successive steps to narrow the region is more efficient than trying to narrow the region with one large cross although this strategy does require additional time. Fourth, studies of the pathophysiology of the disease, which accompanied the genetic studies, were very useful is guiding the search for candidate genes. Finally proving that a candidate gene is the gene encoding the QTL is not easy- simply finding sequence or expression differences is not sufficient proof.

D6. THE GENETIC DISSECTION OF LOW IgE RESPONSE IN THE SJL/J MOUSE INBRED STRAIN.

John Smutko, Jim Vitale, Paul Markel, Bill Paul, Tomo Yoshimoto*, Cyndy Watson*, **Karen J. Moore**. *Genetic Systems, Millennium Pharmaceuticals Inc., 640 Memorial Drive, Cambridge MA 02139. *Laboratory of Immunology, NIAID, NIH, Bethesda, MD.*

The SJL/J inbred mouse strain fails to produce IgE in response to multiple immune stimulation in vivo. This low IgE response is neither due to the B cells being incapable of producing IgE nor due to the T helper cells being incapable of eliciting a normal TH_2 profile. The simplest interpretation of the data is that there is a defect at the priming of TH2 cells. We have taken a genetic approach to understanding the defect in SJL/J mice that drives the low IgE response. Initial genetic analysis revealed that it is the consequence of multiple loci. Subsequent complex trait analysis of the low IgE response has revealed three statistically significant QTLs (LODs of 3.6, 4, and 5) and four suggestive QTLs, two of which show epistatic interaction. We have created interval specific strains carrying the BALB/cJ derived intervals on a SJL/J genetic background in order to further pursue the phenotypic defect in SJL/J and for subsequent gene identification of the causative genes. We will report, for the first time, on the identification of the QTLs and the subsequent phenotypic analysis of the interval specific strains. This presentation will also illustrate potential pifalls of QTL analysis.

D. Quantitative and Mutagenic Traits

Abstracts - Posters

D7. MAPPING OF A LYMPHOMA SUSCEPTIBILITY LOCUS TO MOUSE CHROMOSOME 4.

Joe M. Angel, *Ellen R. Richie. The University of Texas, M.D. Anderson Cancer Center, Science Park-Research Division, Smithville.*

D8. GENETICS OF TPA PROMOTION SUSCEPTIBILITY IN THE MURINE TWO-STAGE SKIN TUMOR MODEL.

Joe M. Angel, *Linda Beltrán, Kevin Hude, John DiGiovanni. The University of Texas, M.D. Anderson Cancer Center, Science Park-Research Division, Smithville.*

D9. CONGENIC MAPPING AND CANDIDATE GENE ANALYSIS OF A SECOND COMPONENT OF THE MHC-LINKED DIABETOGENIC GENE (Idd16) OF THE NOD MOUSE.

Babaya N, *Ikegami H, Kawaguchi Y, Ueda H, *Makino S, Ogihara T. Department of Geriatric Medicine, Osaka University Medical School,Osaka, *Shionogi and Co. Ltd, Shiga Japan.*

D10. GENETIC MAPPING OF A LOCUS INFLUENCING MOUSE SWEET TASTE RESPONSES.

A.A. Bachmanov[1], *D.R. Reed*[2], *M. Inoue*[3], *Y. Ninomiya*[4], *M.G. Tordoff*[1], *R.A. Price*[2], *G.K. Beauchamp*[1,2]. [1]*Monell Chemical Senses Center, Philadelphia, PA, 19104;* [2]*University of Pennsylvania, Philadelphia, PA, 19104;* [3]*Tokyo University of Pharmacy and Life Science, Japan;* [4]*Asahi University, Japan.*

D11. GENETICS OF COMPLEX BEHAVIORS IN THE MOUSE.

Bolivar, V., *Caldarone, B., Flaherty, L. Molecular Genetics Program, Wadsworth Center, P.O. Box 22002, Albany, NY 12201-2002.*

D12. AN INTERESTING CANDIDATE GENE FOR *Lith2*, A QUANTITATIVE TRAIT LOCUS FOR CHOLESTEROL GALLSTONE DISEASE ON CHROMOSOME 19, IS THE CANALICULAR MULTISPECIFIC ORGANIC ANION TRANSPORTER GENE (*Cmoat*).

Guylaine Bouchard, *Hanna C. Chao, Frank Lammert, David Q.-H. Wang, Martin C. Carey, Beverly Paigen. Division of Gastroenterology, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, MA; The Jackson Laboratory, Bar Harbor, ME.*

D13. MAPPING OF QTLS AFFECTING BODY WEIGHT AND FATNESS FROM A HIGH GROWTH SELECTED MOUSE LINE.

G.A. Brockmann[*], *C.S. Haley*[†], *U. Renne*[*], *S.A. Knott*[#], *S. Karle, M. Schwerin*[*]. [*]*Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany;* [†]*Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom;* [#]*Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom.*

D14. *Tmevp2* AND *Tmevp3*, TWO LOCI IN THE TELOMERIC REGION OF CHROMOSOME 10, CONTROL THE PERSISTENCE OF THEILER'S VIRUS IN THE CENTRAL NERVOUS SYSTEM.

Bihl F., Monteyne P., Levillayer F., Brahic M., **Bureau J.-F.** *Unité des Virus Lents (ERS 572 CNRS), Institut Pasteur, Paris, France.*

D15. APPLICATION OF MOUSE GENETICS TO ALZHEIMER DEMENTIA.

George A. Carlson[1], *Costantino Iadecola*[2], *Steven G. Younkin*[3], *Christine Ebeling*[1], *Sherry K. Turner*[1], *Karen K. Hsiao*[2]. [1]*McLaughlin Research Institute, 1520 23rd Street South, Great Falls, Montana 59405;* [2]*Department of Neurology, University of Minnesota, Minneapolis, Minnesota 55455;* [3]*Mayo Clinic Jacksonville, Jacksonville, Florida 32224.*

D16. IDENTIFICATION OF QUANTITATIVE TRAIT LOCI INFLUENCING CAFFEINE 3-DEMETHYLATION IN A C3H/HeJ X APN F2 INTERCROSS.

William L. Casley[1,2], *J. Allan Menzies*[2], *Larry W. Whitehouse*[2], *Thomas W. Moon*[1]. [1]*Department of Biology, University of Ottawa, Ottawa, ON., Canada.* [2]*Therapeutic Products Programme, Health Canada, Ottawa, ON., Canada.*

D17. THE "hot" CHROMOSOMES AND SOME FACTORS, RELATED WITH ITS VARIABILITY IN MICE.

Galina Glazko. *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia.*

D18. GENETIC MOUSE MODELS OF HUMAN NEURAL TUBE DEFECTS.

Muriel J. Harris, *Diana M. Juriloff. Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada.*

D19. GENETIC ANALYSIS OF PLACENTAL DYSPLASIA IN MOUSE INTERSPECIFIC HYBRIDS.

Hemberger, M.[1], *Zechner, U.*[1], *Mayer, W.*[1], *Elliott, R.*[2], *Fundele, R.*[1]. [1]*Max-Planck-Institut für Molekulare Genetik, Berlin, Germany.* [2]*Roswell Park Cancer Institute, Buffalo, NY, USA.*

D20. Pbex1: A QUANTITATIVE TRAIT LOCUS INDUCING PRE-B CELL EXPANSION IN PRECANCEROUS BONE MARROW OF LYMPHOMA-PRONE SL/Kh MICE.

Hiroshi Hiai, *Ling-Min Lu, Reiji Shimada. Department of Pathology and Biology of Disease, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan.*

D21. A LOCUS FOR RADIATION INDUCED GASTROSCHISIS ON MOUSE CHROMOSOME 7.

S. Hillebrandt[1], *C. Streffer*[1], *X. Montagutelli*[2], *R. Balling*[3]. [1]*Institut für Medizinische Strahlenbiologie, Universitäts--klinikum Essen, Hufelandstr. 55, 45122 Essen, Germany.* [2]*Unite de Genetique des Mammiferes, Institut Pasteur, 25 rue du Docteur-Roux, 75724 Paris cedex 15, France..* [3]*Institut für Säugetiergenetik, GSF-Forschungszentrum für Umwelt und Gesundheit, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany.*

D22. MODIFIERS OF MAMMARY TUMOR INITIATION.

Traci Lifsted, Thomas Le Voyer, Max Williams, Kenneth H. Buetow, **Kent W. Hunter.** *Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA, USA.*

D23. TNFa DEFICIENT MICE ARE HIGHLY SUSCEPTIBLE TO TRYPANOSOMA CONGOLENSE INFECTION.

Fuad Iraqi[1], *Kenji Sekikawa*[2], *Alan Teale*[1,3]. [1]*International Livestock Research Institute, P. O. Box 30709, Nairobi, Kenya.* [2]*National Institute of Animal Health. Tsukuba, Japan.* [3]*Current address, Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK.*

D24. MARKER ASSISTED INTROGRESSION OF MULTIPLE UNLINKED QTL: EXPERIMENTAL DESIGNS AND CONSEQUENCES.

O.D. Koudande[1,2], **F. Iraqi**[1], *J. van Arendonk*[2], *A.J. Teale*[1,3]. [1]*International Livestock Research Institute (ILRI), P.O, Box 30709 Nairobi - Kenya.* [2]*Wageningen Institute of Animal Science (WIAS), P.O.Box 338, 6700 AH Wageningen,The Netherlands.* [3]*Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK.*

D25. FINE MAPPING OF TRYPANOSOMIASIS RESISTANCE QTLS IN MICE USING ADVANCED INTERCROSS LINES.

F. Iraqi[1], *S. Kemp*[3], *A. Teale*[1,2]. [1]*International Livestock Research Institute (ILRI), P. O. Box 30709, Nairobi, Kenya.* [2]*Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK.* [3]*School of Biological Sciences, Donnan Labs, University of Liverpool, Liverpool L69 7ZD, UK.*

D26. A LOCUS ON CHROMOSOME 7 DETERMINES MYOCARDIAL CELL NECROSIS AND CALCIFICATION (DYSTROPHIC CARDIAC CALCINOSIS) IN MICE.

Boris T. Ivandic[1,2], *Jian-Hua Qiao*[1,2], *Dietrich Machleder*[1,2], *Feng Liao* [1,2], *Thomas A. Drake* [3], *Aldons J. Lusis* [1,2]. [1]*Division of Cardiology, Department of Medicine,* [2]*Department of Microbiology and Molecular Genetics and Molecular Biology Institute,* [3]*Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095-1679.*

D27. A MOUSE MODEL OF A GENETICALLY COMPLEX BIRTH DEFECT - CLEFT LIP.

Diana M. Juriloff, *Muriel J. Harris, Carolyn J. Brown. Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada.*

D28. QUANTITATIVE TRAIT LOCI ANALYSIS OF CHOLESTEROL GALLSTONE FORMATION IN MICE ESTABLISHES THE BILE SALT EXPORT PUMP GENE (*Spgp*) AS CANDIDATE FOR THE MAJOR GALLSTONE GENE (*Lith1*).

Frank Lammert[1,3], *David Q.-H. Wang*[3], *Valerie Lecureur*[2], *John Schuetz*[2], *David R. Beier*[3], *David E. Cohen*[3], *Martin C. Carey*[3], *Beverly Paigen*[4]. [1]*Department of Medicine III, University of Technology, Aachen, Germany;* [2]*Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN;* [3]*Brigham and Women's Hospital, Department of Medicine, Harvard Medical, Boston, MA;* [4]*The Jackson Laboratory, Bar Harbor, ME.*

D29. QUANTITATIVE INHERITANCE OF GLOMERULOSCLEROSIS IN MICE.

Oliver Lenz [1,3], *Feng Zheng*[1], *Jose Vilar*[2], *Sophie Doublier*[1], *Enrico Lupia*[1], *Susanne Schwedler*[1], *Liliane J. Striker*[1], *Gary E. Striker*[3]. [1]*Renal Cell Biology Laboratory, Division of Nephrology, Department of Medicine, University of Miami School of Medicine, Miami, FL;* [2]*INSERM U319, Paris, France;* [3]*IVAX Research Institute, Miami, FL*

D30. IDENTIFICATION AND CLONING OF GENES INVOLVED IN REGULATING THE RESPONSE TO TUMOR NECROSIS FACTOR IN MICE.

Ben Wielockx, Geoffrey Hammond, Peter Brouckaert, Rosemary Elliott, **Claude Libert.** *Mouse Molecular Genetics Group, VIB Department Molecular Biology, Molecular Pathophysiology and Experimental Therapy Unit (B.W., P.B., C.L.); London Regional Cancer Center, London, Ontario, Canada (G.H.), Roswell Park Cancer Institute, Buffalo, NY (R.E.).*

D31. GENOME WIDE SCAN FOR MODIFIERS OF BSE SUSCEPTIBILITY.

K. Manolakou[1], *M. Bruce*[2], *I. Jackson*[1]. [1]*MRC Human Genetics Unit, Edinburgh,* [2]*Neuropathogenesis Unit, University of Edinburgh.*

D32. THE INFLUENCE OF GENETIC MODIFIERS ON THE LIPOPROTEIN METABOLISM AND SUSCEPTIBILITY TO ATHEROSCLEROSIS OF APOE3LEIDEN MICE.

Corina J.A. Moen[1], *Patrick J.J. van Gorp*[1], *Marion J.J. Gijbels*[1], *Joram de Kraker*[1], *Rune R Frants*[1], *Louis M. Havekes*[2], *Marten H Hofker*[1]. [1]*Dept. of Human Genetics, Leiden University Medical Centre,* [2]*TNO-PG, Gaubius Laboratory, Leiden, The Netherlands.*

D33. TOWARDS PHYSICAL MAPPING OF HYPERACTIVITY QTL IN THE WKHA RAT.

Moisan MP, *Courvoisier H, Cook MN, Durier M, Mormède P. INSERM-INRA Institut François Magendie Bordeaux France.*

D34. LINKAGE OF *Uvs1*, A GENE CONTROLLING SUSCEPTIBILITY TO UV-INDUCED IMMUNOSUPPRESSION, TO CHROMOSOME 6 IN THE MOUSE.

Karen Clemens, Neha Bhatt, Katherine Richardson, **Frances Noonan.**

D35. COMBINATORIAL GENOTYPES PRODUCE EXTREME INFLAMMATORY RESPONSE PHENOTYPES IN RI STRAINS.

L.E. Matesic, A. DeMaio, **R.H. Reeves.** *Johns Hopkins Univ. Schl. Med., Baltimore, MD 21205, U.S.A.*

D36. TWO LOCI ON CHROMOSOME 7 AND 14 INFLUENCE OSTEOSARCOMA SUSCEPTIBILITY IN C3H AND THE 102 MICE AFTER INCORPORATION OF 227THORIUM.

M. Rosemann, *J. Favor, A. Luz, M.J. Atkinson. GSF Institute for Pathology and GSF Institute for Mammalian Genetics, Neuherberg, Germany.*

D37. POSITIONAL CLONING OF THE MOUSE SKELETAL MUTATION, TAIL SHORT (*Ts*).

Kunihiko Shimizu[1,2], *Tuyoshi Koide*[2], *Akihiko Mita*[2], *Kikue Uchida*[2], *Shigeharu Wakana*[3], *Yoshiaki Kikkawa*[4], *Hiromichi Yonekawa*[4], *Hiroki Sasaki*[5], *Toshihiko Shiroishi*[2]. ([1]*Department of Pediatric Dentistry, Nihon University School of Dentistry at Matudo, 2-870-1 Sakaecho-nishi Matudo Chiba 271-8587, Japan,* [2]*Mammalian Genetics Laboratory, National Insutitute of Genetics, Mishima, Shizuoka-ken 411-8540, Japan,* [3]*Central Institute for Experimental Animals, 1430 Nogawa Miyamae-ku Kawasaki 216-0001, Japan,* [4]*Department of Laboratory Experimental Animal Science, The Tokyo Metropolitan Institute for Medical Science, 3-18-22 Honkomagome Bunkyo-ku Tokyo 113-0021, Japan,* [5]*Genetics Division,National Cancer Reserch Insutitute,5-1-1 Tsukiji Chuo-ku Tokyo 104-0045, Japan).*

D38. DYSTONIA AND DEFICIENCY OF SODIUM CHANNEL SCN8A: INFLUENCE OF A MODIFIER LOCUS ON MOUSE CHROMOSOME 3.

Leslie Sprunger, *Andrew Escayg, David D. Lee, Miriam Meisler. Dept. Human Genetics, U. of Michigan, Ann Arbor MI 48109-0618.*

D39. DIFFERENT MODE OF INHERITANCE IN DIABETES-RELATED PHENOTYPES IN SPONTANEOUSLY DIADETIC NSY MICE.

H. Ueda, *H. Ikegami, Y. Kawaguchi, E. Yamato, T. Fujisawa, K. Nojima, N. Babaya, M. Shibata*[*], *T. Ogihara. Department of Geraitric Medicine Osaka University Medical School, Osaka, Japan;* [*]*Aichi-gakuin University, Aichi, Japan.*

D40. GENETIC AND PHYSICAL MAPPING OF *Idd4* tHAT CONTROLS ONSET OF IDDM IN MICE.

Shigeharu Wakana[1], *Toshihiko Shiroishi*[2], *Kazuo Moriwaki*[3], *Chika Maruyama*[1], *Yuka Watanabe*[1], *Tatsuji Nomura*[1]. [1]*Central Institute for Experimental Animals, Kawasaki 216-0001, Japan;* [2]*National Institute of Genetics, Mishima 411-8540, Japan;* [3]*The Graduate Univ. for Advanced Studies, Hayama 240-0193, Japan.*

D41. NEW QTLS THAT MODULATE EYE AND BRAIN DEVELOPMENT: COMPARISON OF RIs, F2s AND AN ADVANCED INTERCROSS.

Robert W. Williams, *Richelle C. Strom, Guomin Zhou, David Airey. The Center for Neuroscience and Department of Anatomy and Neurobiology, Memphis TN 38163.*

D42. THE DROWNING MOUSE STRAIN DERIVED FROM TENASCIN DEFICIENT MICE.

Atsushi Yoshiki, *Moriaki Kusakabe. Division of Experimental Animal Research, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki, Japan.*

D7. MAPPING OF A LYMPHOMA SUSCEPTIBILITY LOCUS TO MOUSE CHROMOSOME 4.

Joe M. Angel, *Ellen R. Richie. The University of Texas, M.D. Anderson Cancer Center, Science Park-Research Division, Smithville.*

Much epidemiological evidence has established environmental factors as the major cause of cancer. These include both chemical and physical carcinogens as well as viruses. Susceptibility to these carcinogens in the general population is a function of multiple, poorly penetrant tumor susceptibility genes that modify the propensity to tumor development. These genes are involved in DNA repair, immune response, carcinogen metabolism and cellular proliferation, differentiation and death. The mapping and isolation of such low penetrance genes in humans is complicated by the multiplicity of unlinked loci involved. This, together with the absence of clear cut familial inheritance patterns, necessitates the development of animal models for the identification of genetic mapping of tumor susceptibility loci.

While animal studies have identified tumor susceptibility and resistance loci in a number of tumor models such as colon, lung, liver and skin, little is known about the genes that modulate susceptibility to carcinogen-induced lymphomas. Strain differences in susceptibility to N-methyl-N-nitrosourea (MNU) induction of thymic lymphomas have been known for years. Previous studies of MNU susceptibility to lymphoma induction in genetic crosses of sensitive AKR/J with resistant C57L/J mice suggested that susceptibility is a multigenic trait. One MNU susceptibility locus (*Tlag1*) was mapped to mouse chromosome 7. This linkage has been confirmed in genetic crosses of AKR and albino deletion mutant mice. Tumor incidence data from AKXL recombinant inbred (RI) mice suggest that at least two additional MNU susceptibility loci segregate in these crosses. Several lines of evidence suggest that a second MNU susceptibility locus maps near *Cdkn2a* on mouse chromosome 4. Analysis of backcross mice homozygous for AKR alleles in the *Tlag1* region of chromosome 7 revealed a significant increase in MNU susceptibility with inheritance of the C57L allele of markers mapping to this region of chromosome 4. In addition, results from tumor studies of AKXL RI strains homozygous for the AKR alleles in the Tlag1 region of chromosome 7 are consistent with the mapping of a second MNU susceptibility locus to chromosome 4. Finally, analysis of tumor DNAs from backcross mice heterozygous for chromosome 4 markers near *Cdkn2a* revealed loss-of-heterozygosity in approximately 8% of the tumors analyzed. These observations, taken together with published reports by others that susceptibility to radiation- and virus-induced lymphomas and pristane-induced plasmacytomas is associated with this region of chromosome 4, suggest that one or more genes important for hematopoietic tumor development maps to the central region of chromosome 4. (Supported by M.D. Anderson Cancer Center Core grant CA16672 and NIEHS Center grant ES07784.)

D8. GENETICS OF TPA PROMOTION SUSCEPTIBILITY IN THE MURINE TWO-STAGE SKIN TUMOR MODEL.

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Cancers fall into two major categories: inherited cancer syndromes and sporadic cancers. The pattern of hereditary cancer is consistent with the effect of a single, highly penetrant autosomal dominant tumor suppressor gene. Epidemiological data has established that inheritance of mutated tumor suppressor genes accounts for only a small proportion of the overall incidence of cancer. Environmental factors are the major cause of cancer and epidemiological data suggest that susceptibility to these carcinogens in the general population is a function of multiple poorly penetrant tumor susceptibility genes that modify the propensity to tumor development. These genes are involved in DNA repair, immune response, carcinogen metabolism and cellular proliferation, differentiation and death.

The mouse skin model is one of the most widely used models for chemical carcinogenesis studies. Genetic differences in susceptibility of mice to two-stage skin carcinogenesis have been known for many years. SENCAR, LACA, CD-1, C3H and DBA are much more sensitive than BALB/c and C57BL/6 mice when treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) as the promoter. The major contribution to susceptibility appears to be at the level of tumor promotion. Analysis of genetic crosses of susceptible DBA/2 or C3H with resistant C57BL/6 mice indicated that susceptibility to TPA promotion is a multigenic trait displaying incomplete dominance. A genome scan of (C57BL/6 x DBA/2)F_1 x C57BL/6 mice scored for TPA promotion susceptibility revealed a TPA promotion susceptibility locus (*Psl1*) mapping to mouse chromosome 9. Further analysis of (C57BL/6 x DBA/2)F_2 and BxD recombinant inbred mice confirmed this linkage and suggested that this locus maps between *D9Mit35* and *D9Mit20*. Several candidate genes mapping to this region of chromosome 9 are being analyzed for their role in TPA promotion of skin tumors. Furthermore, a second promotion susceptibility locus has been mapped to mouse chromosome 10. Preliminary data suggest that this locus influences tumor multiplicity. Studies are underway to confirm the mapping of this TPA promotion susceptibility locus. (Supported by ES08355, M.D. Anderson Cancer Center Core grant CA16672 and NIEHS Center grant ES07784.)

D9. CONGENIC MAPPING AND CANDIDATE GENE ANALYSIS OF A SECOND COMPONENT OF THE MHC-LINKED DIABETOGENIC GENE (Idd16) OF THE NOD MOUSE.

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Type 1 diabetes is caused by autoimmune destruction of insulin-producing b cells of the pancreas. In the nonobese diabetic (NOD) mouse, an animal model of type 1 diabetes, strong susceptibility gene has been mapped to the MHC region on chromosome 17. By using a congenic NOD mouse strain that possesses a recombinant MHC from a diabetes-resistant sister strain, the CTS mouse, we have previously mapped a new susceptibility gene (*Idd16*) to the <11-centiMorgan segment of chromosome 17 adjacent to, but distant from class B, A and E genes (*Idd1*). To further localize *Idd16*, three recombinants within *Idd16* region have been identified, and congenic lines with these recombinants have been monitored for the incidence of type 1 diabetes in comparison with parental NOD strain and previous congenic strain. One recombinant had recombination breakpoint at 1cM proximal to that in the previous congenic line, yet the incidence of type 1 diabetes was still low as compared with the NOD parental strain. These data localized *Idd16* to the <10.2 cM segment of chromosome 17 adjacent to *Idd1*. Since the tumor necrosis factor (TNF) a gene, a candidate gene for type 1 diabetes, is located within the *Idd16* region, the complete nucleotide sequences of coding and 5'-regulatory region of the *TNFa* gene were determined in NOD and CTS mice. Several nucleotide substitutions were found in the *TNFa* gene among NOD, CTS and control strains, but none of them were in cis-elements or exons. These results localized *Idd16* to the <10.2 cM region of the MHC and suggested that *TNFa* is unlikely to be the candidate for *Idd16*.

D10. GENETIC MAPPING OF A LOCUS INFLUENCING MOUSE SWEET TASTE RESPONSES.

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Compared with 129/J (129) mice the C57BL/6ByJ (B6) mice have greater avidity for certain sweeteners and more intensive gustatory nerve responses to them. In order to conduct linkage analysis, we bred the B6 x 129 F2 hybrids, tested their responses to several sweeteners using two-bottle preference tests and electrophysiological recordings from the chorda tympani gustatory nerve, and genotyped them using microsatellite (SSLP) markers. We have mapped a locus responsible for most of this strain difference to a short (< 4 cM long) segment of distal chromosome 4 (chr4). This locus probably corresponds to the previously suggested *Sac* locus (Lush et al., Genet. Res. 1995, 66, 167). It has a strong effect on mouse behavioral and neural responses to sucrose, saccharin and D-phenylalanine, all of which taste sweet to humans. We have started development of a congenic strain, which retains the B6 Sac locus on the genetic background of the 129 strain. Based on SSLP marker genotypes on distal chr4, which were characterized in 455 B6 x 129 F2 hybrids, we identified founders of the congenic strain. Each founder had one copy of distal chr4 originating from the 129 strain; the other copy was recombinant, with a short distal segment from the B6 strain and the more proximal part from the 129 strain. These founders and their offspring are being backcrossed to the 129 strain. By now, we have obtained the second backcross generation. In all backcross offspring, SSLP markers on distal chr4 were genotyped, and saccharin preferences in 2-bottle tests were measured. Additionally, in some mice, chorda tympani electrophysiological responses to sweeteners were recorded. The backcross mice that inherited one copy of distal chr4 from the B6 strain had higher saccharin preferences and chorda tympani responses to sweeteners than did mice that inherited both copies of distal chr4 from the 129 strain. The remaining genetic background from the B6 strain will be eliminated in the next few backcross generations using SSLP marker-based selection. This will lead to production of a high sweetener-responsive 129.B6-Sac strain congenic with the low sweetener-responsive 129 strain.

Supported by NIH grants DC00882, DK44073 and DK48095.

D11. GENETICS OF COMPLEX BEHAVIORS IN THE MOUSE.

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In past work, we have identified several chromosomal regions that affect mouse behavior in the contextual memory portion of a fear-conditioning paradigm. Here, the strongest genes were located on chromosomes 1 and 3 while minor effects were noted on chromosomes 7,8, and 9. In an effort to determine whether these genes were governing the mouse's response to fear versus its memory ability, two new behavioral tests were developed. One of these paradigms measures the ability of the mouse to remember a large open space and the other measures the ability of a mouse to remember the food that it has eaten two weeks previously. When the B6, C3H, 129, and DBA/2 strains were surveyed, there were sharp differences between the behavior of these strains in these behavioral tests. Therefore, we have begun backcrosses to determine the genetics behind these traits. These results will be compared to those that we have previously gathered with the fear-conditioning paradigm.

D12. AN INTERESTING CANDIDATE GENE FOR *Lith2*, A QUANTITATIVE TRAIT LOCUS FOR CHOLESTEROL GALLSTONE DISEASE ON CHROMOSOME 19, IS THE CANALICULAR MULTISPECIFIC ORGANIC ANION TRANSPORTER GENE (*Cmoat*).

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Previously we demonstrated that susceptibility to cholesterol gallstone formation between C57L/J susceptible and AKR/J resistant inbred strains was a complex genetic trait. The major quantitative trait locus, *Lith1*, was found on chromosome 2 which we suggested was the canalicular bile salt transporter sister of P-glycoprotein *spgp* (Hepatology 26:358A, 1997). We now extend our genome wide scan to search for the second most important locus, *Lith2*. We used the progeny of (C57LxAKR)F1 backcrossed to AKR for quantitative trait loci (QTL) mapping. Genotypic characterization for polymorphic microsatellites markers spanning the whole genome was performed by polymerase chain reaction. The progeny phenotype was assessed by microscopy for the presence of cholesterol gallstones after feeding the mice a lithogenic diet (15% fat, 1% cholesterol, 0.5% cholic acid) for 56 days. The weight of cholesterol stones were recorded as the quantitative trait. Northern blot analysis was performed on liver poly(A)-RNA hybridized with murine *Cmoat* cDNA. Introgression of the gallstone predisposing alleles from C57L into the AKR background was performed to generate two congenic strains, AKR.L-*Lith1*^{3} and AKR.L-*Lith2*^{3}. Our genome wide scan identified the second most important QTL *Lith2* in the cross between C57L and AKR to be located on chromosome 19, in the region containing *cmoat*, which, like *spgp*, is a canalicular transporter known to influence biliary lipid secretion. Results obtained from Northern blot analysis revealed two *cmoat* mRNA transcripts that were overexpressed in C57L compared to AKR. These transcripts were also overexpressed in the AKR.L-*Lith2*^{3} congenic strain but not in the AKR.L-*Lith1*^{3} congenic. Taken together, these results suggest that *cmoat* might be responsible for the QTL for gallstone susceptibility identified on chromosome 19. Since *spgp* and *cmoat*, the candidate genes for *Lith1* and *Lith2*, are both transporters expressed at the canalicular membrane between the liver and the biliary space, we suggest that genetic polymorphism of canalicular transporters leading to a gain of function might be the causal factor underlying susceptibility to cholesterol gallstone disease.

D13. MAPPING OF QTLS AFFECTING BODY WEIGHT AND FATNESS FROM A HIGH GROWTH SELECTED MOUSE LINE.

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Quantitative trait loci (QTLs) influencing body weight were mapped by linkage analysis in crosses between a high body weight selected line (DU6) and a control line (DUKs). The two mouse lines, which were generated from the same base population and maintained as outbred colonies, differ in body weight by 106% and in abdominal fat weight by 100% at 42 days. A QTL analysis was performed using structured F_2 pedigrees derived from crosses of a single male from DU6 with a female from DUKs. QTLs significant at the genome wide level were mapped for body weight on chromosome *11*, for abdominal fat weight on chromosomes *4*, *11*, and *13*, and for abdominal fat percentage on chromosomes 3 and 4. The strong effect on body weight of the QTL on chromosome 11 was confirmed in three independent pedigrees. The effect was additive and independent of sex, accounting for 21 to 35% of the phenotypic variance of body weight within the corresponding F_2 populations. The test for multiple QTLs on chromosome *11* with combined data from all pedigrees indicated the segregation of two loci separated by 36 cM influencing body weight.

D14. *Tmevp2* AND *Tmevp3*, TWO LOCI IN THE TELOMERIC REGION OF CHROMOSOME 10, CONTROL THE PERSISTENCE OF THEILER'S VIRUS IN THE CENTRAL NERVOUS SYSTEM.

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Susceptibility to persistence of Theiler's virus in the central nervous system varies greatly among strains of mice. This phenotype is controlled by several genes. One of them is the H-2D gene whose haplotype explains, in most cases, the susceptibility of the mice. However, the SJL/J mice are more susceptible than predicted by their H-2^s haplotype. A genetic analysis of a B10.S x (B10.S x SJL/J)F_1 backcross allows us to localize a locus of susceptibility in the telomeric region of chromosome 10 close to the *Ifng* locus. To confirm this localization, a set of SJL/J mice congenic for different part of the B10.S telomeric region of chromosome 10 was established and studied for susceptibility to the persistence of Theiler's virus. Two loci of susceptibility, *Tmevp2* and *Tmevp3*, have been identified in this region. Further experiments have ruled out that the interferon gamma gene, a good candidate, was involved. Our progress towards the positional cloning of *Tmevp3* will be described.

D15. APPLICATION OF MOUSE GENETICS TO ALZHEIMER DEMENTIA.

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Amyloid-ß peptides (Aß) are thought to be critical in the pathogenesis of Alzheimer dementia. These peptides are the major component of senile plaques and mutations in the vicinity of the Aß coding region of the amyloid precursor protein (APP) gene are linked to early-onset familial AD in rare families. Excess APP due to trisomy 21 also leads to AD pathologies in middle age. Overexpression of mutant or wild-type APP transgenes seemed a logical route towards development of mouse models for AD. However, APP overexpression produced dramatically different phenotypes depending on genetic background. For example, concentrations of APP that lead to florid amyloid plaque deposition in outbred transgenic mice cause premature death at approximately 150 days in FVB/N mice and in FVB/N F1 hybrids with several common inbred strains of mice including C57BL/6J and DBA/2J. Crossing FVB/N transgenic mice with CAST/Ei, 129SvJ, or BALB/cByJ confers resistance to the lethal effects of APP overexpression. To identify genes responsible for susceptibility to APP, we are analyzing (CAST x FVB)F2 and (CAST x FVB)F1 x FVB backcross offspring. Survival data from the F2 cross is complete; 67 of 212 F2 offspring (31%) died prematurely. Similar results are coming from the backcross, suggesting that more than one gene is involved. Microsatellite genotyping is underway.

The cause of APP-induced premature death is unknown, but APP overexpression leads to a profound impairment in endothelium-dependent regulation of the cerebral microcirculation. APP transgenic mice are hyporesponsive to vasodilators whose action is mediated through endothelium but respond normally to endothelium-independent vasodilators. Cerebrovascular dysfunction was not seen in double transgenic mice overexpressing APP and superoxide dismutase-1 (SOD1), indicating the involvement of reactive oxygen species. SOD1 transgene overexpression also protects against APP-induced premature death.

D16. IDENTIFICATION OF QUANTITATIVE TRAIT LOCI INFLUENCING CAFFEINE 3-DEMETHYLATION IN A C3H/HeJ X APN F2 INTERCROSS.

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The metabolism of caffeine is used as a clinical marker for the determination of *in vivo* cytochrome P450 CYP1A2 activity in humans. CYP1A2 is involved in the metabolism of a number of drugs as well as the activation of several environmental procarcinogens. Conflicting data from caffeine assays have suggested a uni-, bi- or tri-modal distribution of CYP1A2 activity in human populations, although no evidence for a genetic expression polymorphism linked to the CYP1A2 locus has been demonstrated. We have investigated the possibility that inbred mouse strains might be useful in the identification of genetic determinants of variable CYP1A2 expression, using caffeine 3-demethylation (C 3-D) as a quantitative phenotypic marker. We compared the plasma levels of caffeine and the 3-demethylated metabolite, 1,7-dimethylxanthine, in six common inbred strains and one inbred strain (APN) derived in our laboratory from outbred Swiss-Webster mice. Significant variations between a number of the common strains were observed, all of which produced significantly higher C 3-D indices than the APN strain. The observed difference between APN and C3H/HeJ strains was reflected in elevated hepatic microsomal enzyme activity, CYP1A2 immunoreactive protein levels and hepatic steady state CYP1A2 mRNA levels. CYP1A2 mRNA levels were positively correlated with C 3-D values. Phenotypic data were obtained for an F2 intercross of C3H/HeJ X APN and the phenotypic extremes were genotyped at marker loci which achieved complete coverage of the genome. A number of putative QTLs were identified, including highly significant linkage to the map position of the *Cyp1a2* gene on chromosome 9.

D17. THE "hot" CHROMOSOMES AND SOME FACTORS, RELATED WITH ITS VARIABILITY IN MICE.

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In genetic variability it is accepted to allocate some levels of changes in genetic materials, - genome, chromosome and gene ones. Early was shown, that nucleotide replacement in sequences of structural genes are much distinguished in frequency and for each mutational spectrum there are some various classes, consisting from specific frequency and type of replacements. It is known, that one of the factors of influence on occurrence of such various classes appear nucleotide context - presence of some nucleotides, influencing on the mutability of certain site in nucleotide sequence [1].

It is possible to expect, that similarly to existence of "hot points" of mutagenesis in nucleotide sequences on gene level, there are "the hot points" of variability on chromosome level - individual chromosome with increased frequency of involving in mutation events of this level of genetic variability [2]. The influences to occurrence of the similar "hot points" - "hot chromosomes" can render as "context" of this level of variability. The given work is devoted to finding out the possible factors of such "context" and degree of their influence on the level of variability of individual chromosomes at some laboratory mouse lines.

The analysis of the order of the chromosome involving to the interchromosome associations between heterologous chromosomes (for type of Robertsonian translocations - RB), the frequencies of the associations betweeen individual homologous chromosomes (identificated by G-bands) in the 28 cell populations in relation with three factors - their origin from different mouse lines (CBA/Ca, C3H/He, C57BL/6, BALB/c), direction of cytodifferentiation (fibroblasts and leukocytes) and the stage of its disruption (minimal, maximal and medial tumorigenicity) was carried out. The presence of the interrelations between individual chromosome associations and the genetic and cytological cell characteristics with the uses of the different mathematical methods of analysis was evaluated. The influencies of all three factors on the frequencies of individual chromosome involving in RB were revealed. The compexes of the "context" of the chromosome level of the genetic variability is discussed.

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D18. GENETIC MOUSE MODELS OF HUMAN NEURAL TUBE DEFECTS.

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More than 40 spontaneous or targeted single-locus, Mendelian, mutations cause neural tube closure defects, exencephaly or spina bifida, in mice. A review of these mutations shows that most have major defects in other developmental systems and many are lethal before birth. In contrast, most cases of neural tube defects among human newborns are non-syndromic and have a genetically complex etiology. This situation leads to two considerations – that in these mutants exencephaly may be a nonspecific symptom of a dying embryo, and that the human homologues of these mutants may not comprise a significant proportion of human neural tube defect cases.

At least four mouse strains have exencephaly (SELH/Bc, MT/HokIdr, NZW-xid, BXA-2/Pgn) in 10% to 20% of newborns and one (the "curly tail" strain) has 15-20% spina bifida. Affected embryos in these strains survive to birth and have no other independent developmental defects. The liabilities in SELH/Bc and curly tail have been studied and shown to be genetically complex, but the genes are not yet identified.

As in human anencephaly, females are in excess among exencephalic fetuses in the SELH/Bc, curly tail, and NZW strains, and in the Macs and Trp53 null mutants. This pattern suggests a fundamental difference between males and females at the time of neural tube closure.

It is well known that up to 70% of human neural tube defects can be prevented by maternal periconceptional folate supplementation, but the mechanism of this effect is not known. One of the hallmarks of the NTD mouse mutants and strains is the numerous preventative responses to a variety of nutrients – specific nutrient/genotype interactions. Among these, exencephaly in the SELH strain shows no response to folic acid supplementation, but has a 3-fold higher frequency in mothers fed Purina Diet #5015 than Purina Diet #5001. Studies are in progress to identify the specific dietary agent involved.

Exencephaly in the SELH/Bc mouse strain, the model developed and studied in our laboratory, appears to be caused by the joint effect of 2 or 3 unlinked loci. One of these loci appears to be in mid-Chromosome 13, which includes candidate genes such as *Msx2*, *Neurod3*, *Otp*, and *Tcfap2a*. Further mapping is in progress. When identified, these genes for NTD risk in mice will provide candidate genes to test for association with risk for NTD in humans. Developmentally, all SELH embryos leave out one normal step in cranial neural tube closure (initiation of closure at the forebrain-midbrain boundary - Closure 2), but in 80-85% closure is completed by the compensatory extension of closure from the most rostral end of the neural tube (Closure 3). In 15-20%, the midbrain neural folds never elevate, leading to exencephaly. A question for the future is whether the embryos of the other susceptible genotypes that manage to complete cranial neural tube closure (i.e., become normal) also lack the Closure 2 initiation site and complete closure by extension from the most rostral site.

(Research funded by the Medical Research Council of Canada.)

D19. GENETIC ANALYSIS OF PLACENTAL DYSPLASIA IN MOUSE INTERSPECIFIC HYBRIDS.

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Placental dysplasia, caused mainly by over growth of the spongiotrophoblast, was observed in interspecific hybrids between *Mus musculus (mus)* and *Mus spretus (spr)*. Genetic mapping analysis resulted in a significant linkage for the whole proximal and central part of the X chromosome. Congenic mouse strains were generated to reveal critical intervals within this large chromosomal region. One of these strains retained a *spr* derived part on the X chromosome between 11.5 and 31.7 cM. This line clearly developed enlarged placentas, although placental hyperplasia was less pronounced compared to F1 females. Further dissection of this portion resulted in the loss of weight increase in each of the subcongenic strains. However, it could be shown that at least in two of these subcongenic lines the spongiotrophoblast layer was still enlarged. Interestingly, the *spr* derived regions of these subcongenics did not overlap. Further investigations will have to show which genetic mechanism causes placental dysplasia in mouse interspecific crosses.

D20. Pbex1: A QUANTITATIVE TRAIT LOCUS INDUCING PRE-B CELL EXPANSION IN PRECANCEROUS BONE MARROW OF LYMPHOMA-PRONE SL/Kh MICE.

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The SL/Kh mouse is an inbred strain spontaneously developing pre-B lymphomas at ~100% by 6 months of age. Our previous study revealed that endogenous ecotropic provirus *Emv-11* (Chr. 7) and a dominant SL/Kh allele of *Esl-1* closely linked to MHC (Chr. 17) are essential for pre-B lymphomas. SL/Kh mice showed a transient polyclonal expansion of pre-B cells in bone marrow (BM) at 4-6 weeks of age followed by monoclonal growth of pre-B cells starting at about 3 months of age. Radiation chimera experiment showed that such expansion is a genetic property of SL/Kh BM stem cells rather than their microenvironments. Furthermore, such expansion is not prevented by suppression of endogenous MuLV by crossing with Fv-4R mice or by administration of maternal resistance factor (anti-MuLV gp70 antibody). To map the gene inducing the pre-B cell expansion, genetic analysis was performed in 159 F2 intercross of SL/Kh and NFS/N and 338 backcross to SL/Kh, using the percentage of pre-B cells in BM lymphocytes as a quantitative parameter. Quantitative trait locus (QTL) analysis revealed a highly significant QTL with LOD score 22.7 in F2 and 10.68 in backcross mice on chromosome 3. This QTL, designated as Pre-B expansion-1 (*Pbex1*), colocalizes with *LEF1* (lymphoid-enhancer factor-1) encoding a lymphoid-specific transcription factor and plays critical roles in the early B cell development. It is still unclear whether the polyclonal expansion is an essential requirement for pre-B lymphomas to occur. Similar perturbed differentiation of pre-B cells, however, is seen in other pre-B lymphomagenesis models by Abelson MuLV or by Emu-myc transgene.

D21. A LOCUS FOR RADIATION INDUCED GASTROSCHISIS ON MOUSE CHROMOSOME 7.

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Gastroschisis (abdominal wall defects) occurs with a high frequency in the mouse inbred strain HLG compared to C57BL/6J mice. The risk of gastroschisis increases significantly after exposure to irradiation with X-rays during preimplantation development and follows a recessive mode of inheritance for the HLG susceptibility alleles. The frequency of gastroschisis after backcrossing is consistent with the two or three loci control the susceptibility to radiation-induced gastroschisis (Hillebrandt et al. 1996).

We have used the genome wide microsatellite typing of BC1 mice to chromosomally map this trait. Significant linkage for a locus responsible for radiation induced gastroschisis (*Rigs1*) was found in a region of mouse chromosome 7. One marker (*D7Mit315*) on Chromosome 7 showed a highly significant linkage with the phenotype (p=0.00020). This indicates that at least one of the loci responsible for the development of radiation-induced gastroschisis maps to Mouse chromosome 7 close to this marker.

D22. MODIFIERS OF MAMMARY TUMOR INITIATION.

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The inherited component of breast cancer was originally observed more than 100 years ago and has been confirmed by the identification of two major susceptibility genes, *BRCA1* and *BRCA2*. Women with germline mutations in these loci have a greatly enhanced risk of breast cancer. The likelihood of women carrying mutations in *BRCA1* approaches approximately 80% by the age of 70 and carriers are also at increased risk for ovarian cancer. Approximately 70% of breast cancer families can be account for by these high susceptibility genes and between 10 - 15% of breast cancer in the general population.

Although the discovery of these high susceptibility genes is important for understanding the genetic basis of breast cancer many important questions remain to be answered. The clinical expression of BRCA1 has been shown to vary both between families and within families. Some women may develop breast cancer in early in life, while family members bearing the same mutation remain unaffected until their seventies. The variable penetrance and age-at-onset observed between individuals bearing the same mutations suggests that there must be additional factors that influence the development of the disease. At present, however, little is known about breast cancer modifier genes and how they interact with the major susceptibility genes. Due to the genetic heterogeneity of the human population and interactions with uncontrolled environmental influences, identification of modifiers in human populations can be a difficult task.

To identify the genetic factors that influence the age of onset of mammary tumor intitiation our laboratory studies the the FVB/N-TgN(MMTVPyMT) transgenic mouse. This animal bears the polyoma middle T antigen whose expression results in the development of palpable synchronous multifocal tumors at approximately 60 days of age. The mammary tumors replicate many of the biochemical and histological events of some breast cancers. To identify inbred strains bearing genes that modify the dominantly expressed tumor phenotype, we have performed a mouse strain survey by breeding this transgenic animal to 27 different inbred strains from different branches of the mouse phylogenetic tree and determining the average age of tumor induction. The primary tumors in the F_1 progeny of two inbred strains, C58/J and I/LnJ, were detected approximately two and a half weeks earlier than the FVB/N transgenic parent strain. In addition, the F_1 progeny of 6 strains, SWR/J, AKR/J, BUB/BnJ, ST/J, KK/HiJ and MOLF/Ei, developed palpable tumors approximately 1 to 5 weeks later than the FVB/N-TgN(MMTVPyMT). These data strongly suggest the presence of genetic modifiers of mammary tumor initiation in this model system. We are currently developing backcrosses to map the loci responsible for the alteration in mammary tumor latencies.

D23. TNFa DEFICIENT MICE ARE HIGHLY SUSCEPTIBLE TO TRYPANOSOMA CONGOLENSE INFECTION.

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The murine Trypanosoma congolense infection resistance QTL, *TIR1*, is located on MMU17 and encompasses the *TNFa* locus. For a number of reasons, *TNFa* is currently a candidate for *TIR1*, away which is the fact that *TNFa* would appear to be a factor in determining susceptibility to protozoal infections. In recent studies we have found correlation between *TNFa* haplotypes of different mouse strains and its susceptibility to trypanosomiasis, and difference expression of *TNFa* in resistant and susceptible mouse strains. As an additional way to confirm the candidacy of *TNFa* for *Tir1*, we have studied the response of *TNFa* deficient mouse that was developed from resistance mouse strain (C57BL/6) to Trypanosoma congolense infection. Hemizygote *TNFa* deficient mice with the resistant or susceptible mouse background were also developed and challenged. Two resistant and susceptible mouse strains, C57BL/6 and A/J respectively, were challenge for control. *TNFa* deficient mouse was shown to be highly susceptible to the challenge with mean survival time of 36 days. It was shown to be more susceptible than the susceptible control mouse A/J, which had survival time of 51 days. The mean survival time of the resistant mouse strains, C57BL/6, was shown to be 95 days. The hemizygote *TNFa* deficient mouse with the resistant background was shown to be more susceptible than the resistant strain with mean survival time of 76 day, while the hemizygote with the susceptible background was shown to be slightly more resistant than the susceptible mouse strain with mean survival time of 84 days. This data support our proposal that *TNFa* is an important gene for the mouse resistance to trypanosomiasis.

D24. MARKER ASSISTED INTROGRESSION OF MULTIPLE UNLINKED QTL: EXPERIMENTAL DESIGNS AND CONSEQUENCES.

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Marker assisted introgression (MAI) of multiple unlinked QTLs may be achieved in several waysachieved in several ways. In this report, three unlinked QTLs are under consideration which contribute to resistance to trypanosomiasis in the C57BL/6J donor mouse strain, and which are revealed in crosses with the A/J recipient strain. Using this mouse model, five paths (or options) have been examined, each one starting from BC1 animals. Within the BC1 resulting from the cross between the F1 (A/J x C57BL6) and the recipient strain, the potential genotypes expected are: heterozygous at the 3 QTLs (1/8 of the offspring), homozygous (animals non carrier) at the 3 QTLs (1/8 of the offspring); heterozygous at 2 QTLs (3/8 of the offspring) and heterozygous at 1 QTL (3/8 of the offspring). This study shows that choice of introgression strategy can be influenced, among other things, by the number of generations needed to complete the process, as well as by the number of animals to be genotyped during the process In these cases, when using only donor males through the BC breeding phases, together with multiple mating and littering, developing any two double carrier lines appears to be the most efficient design. In the final stage, intercrossing within and between lines gives rise to animals homozygous for the resistance allele(s) at 1, 2 or 3 loci. This provides the opportunity to study the individual and combined effects of three QTLs in all possible combinations on a given genetic background.

D25. FINE MAPPING OF TRYPANOSOMIASIS RESISTANCE QTLS IN MICE USING ADVANCED INTERCROSS LINES.

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High resolution mapping of quantitative trait loci (QTL) is an important step towards positional cloning or positional candidate gene identification. Recently, the use of advanced intercross lines (AIL) for dissection and fine mapping of QTL was described (Darvasi and Soller, 1995). In this study we have used AILs to fine map resistance to trypanosomaisis caused by Trypanosoma congolense infection in mice. Previously, we mapped 3 QTL, *Tir1*, 2 and 3, located on chromosomes 17, 5 and 1 respectively, which explain almost all of the genetic variance in F2 populations produced by crossing the resistant C57BL/6 and two susceptible strains, A/J and BALB/c. The QTLs were mapped to regions spanning 20-40 cM. Following mapping in the F2s, we developed 2000 F6 C57BL/6xA/J advanced intercross mice. The 2000 F6 mice were challenged with *T. congolense*, and 1750 of them were infected and taken for analysis. 66 Microsatellites located within the 3 QTLs at intervals of 3 cM were genotyped on mice representing phenotypic extremes. Thus the 200 mice which were the first to succumb and the 200 which were the last to succumb were genotyped. Genetic and phenotypic data were analysed with the MAPMAKER/EXP/QTL programmes and each of the QTLs was mapped to a 5-10 cM. Preliminary results indicate that the chromosome 17 QTL, *Tir1*, comprises at least two loci with LOD scores of 13 and 15 separated by 0.5 cM. Similar results were obtained with the chromosome 1 QTL, *Tir3*, where indications of two loci, each mapping to a separate 5 cM region with LOD scores of 8 and 4 separated by 4 cM, were found. The chromosome 5 QTL, *Tir2*, was mapped to 5 cM with a LOD score of 3. Currently more mice are being genotyped to increase mapping resolution and additional fine mapping of each QTL to a region of 0.5 cM is ongoing in interval specific congenic strains (ISCSs).

D26. A LOCUS ON CHROMOSOME 7 DETERMINES MYOCARDIAL CELL NECROSIS AND CALCIFICATION (DYSTROPHIC CARDIAC CALCINOSIS) IN MICE.

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Dystrophic cardiac calcinosis, an age-related cardiomyopathy that occurs among certain inbred strains of mice, involves myocardial injury, necrosis, and calcification. Using a complete linkage map approach and quantitative trait locus analysis, we sought to identify genetic loci determining dystrophic cardiac calcinosis in an F_2 intercross of resistant C57BL/6J and susceptible C3H/HeJ inbred strains. We identified a single major locus, designated *Dyscalc*, located on proximal chromosome 7 in a region syntenic with human chromosomes 19q13 and 11p15. The statistical significance of *Dyscalc* (logarithm of odds score 14.6) was tested by analysis of permuted trait data. Analysis of BxH recombinant inbred strains confirmed the mapping position. The inheritance pattern indicated that this locus influences susceptibility of cells both to enter necrosis and to subsequently undergo calcification.

D27. A MOUSE MODEL OF A GENETICALLY COMPLEX BIRTH DEFECT - CLEFT LIP.

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Cleft lip (formerly "hare-lip") with or without cleft palate, "CLP", is a common human birth defect with a genetically complex, multifactorial etiology. It is often confused with isolated cleft palate, "CP", a defect of developmental and genetic etiology demonstrably distinct from CLP in most affected families. In mice, numerous targeted mutations cause CP, but none so far have caused CLP. In contrast to other birth defects, CLP is almost never seen in most inbred strains. There is only one known genetic CLP trait in mice, present in all "A/-" strains (e.g., A/J, A/HeJ, A/WySn) and in strains with A/- strain ancestry (CL/Fr, AXB-6/Pgn). CLP is a developmental threshold trait; consequently not all individuals with the genetic liability will express the trait - 5% to 30% of fetuses and newborns in these strains have CLP and die soon after birth.

Similar to its human counterpart, the CLP of the A/- strains has a genetically complex, multifactorial etiology. The embryological deficits in maxillary prominence size that lead to CLP also appear similar, and the CLP of the A/- strains appears to be an excellent model that can point to the genetic pathways involved in causation of human CLP.

A congenic strain approach previously enabled the mapping of a recessive A/- gene, *clf1*, essential to risk of CLP; it is on Chr 11 linked to the *Hox* gene cluster. The presence in the A/- strains of a second essential CLP risk gene, *Clf2*, probably semidominant, was also demonstrated. The two-locus interacting genotypes that the model predicts to be the cause of risk of CLP in embryos are: *clf1/clf1 Clf2/+* and *clf1/clf1 Clf2/Clf2*. Current studies based on affected backcross embryos from a cross of C57BL/6J and A/WySn, have confirmed and refined the linkage mapping of *clf1* relative to SSLPs. Some candidate loci have been excluded by amplifying from YACs that map outside the defined *clf1* region. Mapping of *Clf2* is based on the backcross embryo panel. Hypothesis-driven tests of 3 genomic regions have produced a strong candidate region for linkage of *Clf2* ($P<.05$ after Bonferroni correction) and increases to the sample are in progress.

Although genotype of the embryo sets up risk of developing CLP, much of the genetic complexity of inheritance of risk of CLP in mice originates in genetically determined maternal effects which can modulate the risk in embryos 3-fold. Genetic variation causing these maternal effects in mice appears to be very common; the strongest risk is associated with the A/WySn and CL/Fr maternal genotypes. There is evidence of genetic maternal effects on CLP risk in humans also. Environmental manipulation of maternal physiology offers an attractive strategy for prevention of CLP, and identification of the genetic pathways causing maternal effects is therefore important. Projects to map the maternal effect loci are in progress, including the construction of some new "assisted" RI strains between A/WySn and C57BL/6J.

(Research funded by the Medical Research Council of Canada.)

D28. QUANTITATIVE TRAIT LOCI ANALYSIS OF CHOLESTEROL GALLSTONE FORMATION IN MICE ESTABLISHES THE BILE SALT EXPORT PUMP GENE (*Spgp*) AS CANDIDATE FOR THE MAJOR GALLSTONE GENE (*Lith1*).

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Cholesterol gallstone formation is one of the most common and economically significant digestive diseases, yet the genetic basis of this complex trait is unknown. Among several inbred strains investigated, C57L/J mice displayed high cholesterol gallstone prevalence (>80%), whereas AKR/J mice showed low stone prevalence (<20%) while being fed a lithogenic diet (15% fat, 1% cholesterol, 0.5% cholic acid) for 8 weeks. Herein we describe a search to detect the major lithogenic gene *Lith1*. Quantitative trait loci (QTL) analysis was performed in progeny of (C57LxAKR)F_1 mice backcrossed to AKR mice. Gallstone phenotypes were assessed by polarizing light microscopy of gallbladder bile and gallstones after feeding the lithogenic diet for 8 weeks, using gallstone weight as the quantitative trait. A genome wide scan identified significant (LOD score 4.4) linkage of gallstone susceptibility to a locus on Chromosome 2. Utilizing progeny testing, the gene *Lith1* was subsequently mapped to a 0.2 cM region at *D2Mit56*. The congenic strain AKR.L-*Lith1*^{S} was bred by marker-assisted introgression of the susceptible *Lith1* alleles from C57L into the AKR background. Phenotypic characterization of parental, F_1 and congenic mice was complemented by physical-chemical analysis of gallbladder bile, determination of hepatic biliary lipid secretion rates via an acute biliary fistula as well as Northern and Western blot analyses of hepatic *Spgp* expression. Because in gallstone-susceptible mice, these functional studies demonstrated increased hepatic secretion rates of all biliary lipids (cholesterol, bile salts and lecithin), we explored whether genes encoding hepatic canalicular transporters for biliary lipids map to *Lith1*. Indeed the gene encoding the bile salt export pump (Spgp) co-localized with *D2Mit56/Lith1*, in contrast to the genes of the lecithin transporter (Mdr2) and the conjugate export pump (*Cmoat*). Furthermore in comparison to gallstone-resistant AKR mice, strains carrying the susceptible *Lith1* alleles (C57L, AKR.L-*Lith1*^{S}) exhibited striking overexpression of *Spgp* at mRNA and protein level both before and during lithogenic diet feeding. **Summary:** *Lith1* provides an example for efficient and rapid identification of a novel candidate gene for a complex trait by combination of QTL analysis and functional studies. Chromosomal location and differential expression suggest that alterations in the function of the bile salt export pump Spgp are primary pathophysiological events that lead to cholesterol gallstone formation in mice.

D29. QUANTITATIVE INHERITANCE OF GLOMERULOSCLEROSIS IN MICE.

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Glomerulosclerosis, the common terminal event in chronic glomerular diseases such as diabetic nephropathy or IgA nephropathy, is characterized by mesangial expansion and the progressive accumulation of extracellular matrix, and leads to end stage renal disease. The considerable variation in both the risk to develop glomerulosclerosis and the rate of progression in individual patients suggest a role for genetic factors which have not been identified so far. In this study, we investigated the inheritance of glomerulosclerosis using a sclerosis-prone strain, ROP/Le-OsEs1<a>/+Es1<b> (ROP-Os), and a control strain, C3H/HeJ (C3H). We have previously shown that the Os mutation can be used as a tool to reveal the sclerosis-trait at an early age, since only susceptible animals develop progressive glomerular disease by the age of 3 months. To assess the extent of glomerular lesions in each mouse we measured the surface area of the mesangial matrix and the total glomerulus by computer-aided morphometry on PAS stained paraffin sections of Formalin-fixed kidneys. For each section, the ratio mesangial area/total glomerular area (in percent) obtained from 20 randomly chosen glomeruli served as the sclerosis score. While C3H mice did not spontaneously develop glomerulosclerosis (mean score: 9 +/- 1.8, n=10), ROP-Os animals showed severe lesions (mean score: 27 +/- 4.7, n=10). F1-Os/+ animals of the cross C3H X ROP-Os had normal glomeruli (mean score: 7 +/- 1.3, n=10). Thus, we performed a backcross to the ROP-Os/+ strain. BC1-Os/+ mice showed a wide distribution of glomerular lesions (score range: 7.2 - 28.0, mean score: 14.5 +/- 4.4, n=50). According to the formula of Wright, we estimated the number of loci involved in the inheritance of this disease as at least eight. In conclusion, we have demonstrated the quantitative inheritance of glomerulosclerosis in mice. Using this new model, it may be possible to identify genes, which determine the individual risk to develop glomerulosclerosis in the course of chronic glomerular diseases such as diabetic nephropathy or IgA nephropathy.

D30. IDENTIFICATION AND CLONING OF GENES INVOLVED IN REGULATING THE RESPONSE TO TUMOR NECROSIS FACTOR IN MICE.

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Tumor Necrosis Factor (TNF) is a cytokine, produced mainly by macrophages upon trigger with bacterial or viral components. It has a very potential antitumor activity, especially when used in combination with Interferon. However, clinical trials in human patients, and experiments in rodents reveiled that TNF is a powerful inducer of inflammation. Therefore, administration of TNF in the general circulation is currently impossible because of the induction of a lethal Systemic Inflammatory Response Syndrom (SIRS). We are interested in identifying, cloning and eventually applying endogenous factors that counteract the lethal effects of TNF. We have found that several inbred strains of mice display different sensitivity to an intravenous injection of TNF. We found that, compared to C57BL/6J mice, BALB/cJ mice are extremely sensitive while DBA/2J mice are significantly resistant. Using the complete set of 26 BXD recombinant inbred strains and a dose of TNF lethal for C57BL/6 but not for DBA/2 mice, we found that a locus on distal chromosome 12 is closely linked to the TNF resistance observed in DBA/2 mice. QTL analysis of drop in body temperatures and induction of an other cytokine, IL-6, 6 hours after TNF challenge, confirmed the importance of this locus. Backcross experiments starting from several parental strains are underway to identify other loci that could influence TNF-induced lethal SIRS. Genes found in the chromosome 12 locus are the serine protease inhibitor genes (SERPINs) coding for a-1-antitrypsin, Corticostroid Binding Globulin and contrapsin. We are currently studying the role of these genes and are cloning other genes from this locus following family PCR and differential expression approaches. Supported by the ASLK, the IUAP and the FWO. CL and PB are senior research associates with the VIB and FWO respectively and BW holds an IWT fellowship.

D31. GENOME WIDE SCAN FOR MODIFIERS OF BSE SUSCEPTIBILITY.

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The *PrP* gene, on distal chromosome 2, and the *Pid-1* locus, in the D subregion of H-2 on chromosome 17, have been reported to control scrapie and/or CJD susceptibility in mice. Hence there was not a one-to-one relationship between genotype and phenotype suggesting that prion susceptibility might be a complex trait.

The biological timing of BSE induced disease vary between C57BL6 and RIII strains of mice although these strains are apparently identical for the *PrP* gene, which suggests the existence of modifiers responsible for prion susceptibility. A simple model fitted to phenotypic data of a preliminary cross between these strains leads to a rough estimation of three genes controlling BSE susceptibility. In order to identify and map these genes we set up backcrosses between the two strains.

A total of 1200 N1 backcross animals have been challenged intra-cerebrally with BSE inoculum. Currently we are collecting microsatellite genotype data for 376 of these progeny with 80 markers spread through out the genome. The incubation period of the disease (days till death) will be available shortly to perform a QTL analysis on the first batch of genotyped animals. That fraction of the rest 824 N1 backcross animals corresponding to the extremities of the phenotypic distribution, will be genotyped subsequently at those chromosomal regions in which a QTL has been detected, in order to narrow down the confidence intervals.

We will present the phenotypic data of the preliminary cross, the adopted experimental design and genome wide density as well as a summary of the genotypic data collected so far.

D32. THE INFLUENCE OF GENETIC MODIFIERS ON THE LIPOPROTEIN METABOLISM AND SUSCEPTIBILITY TO ATHEROSCLEROSIS OF APOE3LEIDEN MICE.

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The development of atherosclerosis is a complex process in which the lumen of a blood vessel becomes narrowed by cellular and extra-cellular substances to the point of obstruction. The plasma levels of lipoproteins and their composition (cholesterol/triglycerides) are strongly associated with atherosclerosis. Genetic as well as environmental factors play an important role in the development of hyperlipidemia and atherosclerosis.We have focused on apolipoprotein E (APOE), which plays a crucial role in the clearance of lipoproteins from the blood. Several APOE mutations, including the dominant APOE3Leiden variant, are associated with familial dysbetalipoproteinemia (FD). FD is characterized by impaired lipoprotein remnant uptake leading to hyperlipidemia and atherosclerosis. However, the clinical phenotype of gene carriers is highly variable, due to the influence of additional genetic and environmental factors. We use transgenic mice overexpressing human APOE3Leiden to identify these modifying genes. The APOE3Leiden mice carry the transgene on a >99% pure C57BL/6J background (N16th generation) and are highly susceptible to diet-induced hyperlipidemia and atherosclerosis due to a defect in hepatic uptake of chylomicron and VLDL remnant proteins. In contrast to non-transgenic mice, the main lipid change in the APOE3Leiden mice takes place in the VLDL fraction, allowing the analysis of genetic factors involved in VLDL metabolism. Our approach to identify genes involved in lipid metabolism and susceptibility to atherosclerosis is the quantitative trait locus (QTL) mapping. Therefore we studied which inbred mouse strains carry genes that modify the phenotype of the APOE3Leiden strain. We tested female F1 hybrids and backcross mice between the APOE3Leiden mice and 7 different inbred mouse strains (CBA/J, C3H/HeJ, NZB/Orl, FVB/N, 129/Ola, BALB/cByJ, AKR/J) for their plasma lipid levels and susceptibility to atherosclerosis to look for dominant and recessive modifying genes, after feeding the mice several types of diets (Chow, Low Fat/no Cholesterol (LFC), High Fat/high Cholesterol (HFC)). Striking differences in cholesterol and triglyceride levels were found between the APOE3Leiden strain and F1 and/or backcrosses between the APOE3Leiden strain and the inbred strains on the different diets. Notably, FVB carries modifiers that increase the levels of cholesterol as well as triglycerides on all diets. Another promising result is that {129 x (129 x APOE3Leiden)}backcross mice are less susceptible to atherosclerosis after feeding HFC, as compared to the APOE3Leiden strain, while the cholesterol levels were similar. Hence, the 129/Ola mice carry a set of genes preventing atherosclerosis, that differ from the genes that influence cholesterol levels.

D33. TOWARDS PHYSICAL MAPPING OF HYPERACTIVITY QTL IN THE WKHA RAT.

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The etiology of behavioral hyperactivity is very poorly understood because of its heterogeneity. To better understand the neurobiology of hyperactivity we have undertaken a genetic mapping strategy, using inbred rat strains, WKY and WKHA distinct for their low and high activity scores respectively. QTL genetic linkage analysis using 67 polymorphic genetic markers and sixteen phenotypic measures (behavioral and endocrine) on a 196 F2 population detected a major locus on chromosome 8 (Lod score=10, 85% of the genetic variance explained) that influences motor activity but is independent of emotionality (Moisan et al, Nat Genet 14, 471-473 1996). In order to reduce the QTL interval several approaches were attempted: (i) we have constructed a region-specific genomic library by microdissection of rat chromosome 8, (ii) we have tried to do a representational difference analysis between WKHA and partially congenic genomes, (iii) SSCP for candidate genes were tested. So far we have had very limited sucess with these experiments. Only one additional marker was placed in the QTL area and the LOD score of the QTL was increased from 10 to 14. Our efforts are hampered by the low polymorphism rate between WKY and WKHA strains. Thus we have now started to analyse a F2 population from a WKHAxBN cross. We hope to detect again the hyperactivity QTL on this more polymorphic cross. In addition congenic strains between WKY and WKHA are being constructed to study the physiological impact of this locus.

D34. LINKAGE OF *Uvs1*, A GENE CONTROLLING SUSCEPTIBILITY TO UV-INDUCED IMMUNOSUPPRESSION, TO CHROMOSOME 6 IN THE MOUSE.

Karen Clemens, Neha Bhatt, Katherine Richardson, **Frances Noonan.**

Ultraviolet B radiation (UVB, 290-320nm) initiates a dose and wavelength dependent down regulation of cell-mediated immunity in mouse and in man. This form of immunosuppression appears to be a critical event in UV carcinogenesis, preventing the immunologic rejection of antigenic UV-induced skin cancers. We previously demonstrated that susceptibility to UV-induced immunosuppression was controlled by 3 interacting genes, two autosomal genes, *Uvs1* and *Uvs2* and an X-linked gene *Uvs3*. In the current study, we set out to establish genetic linkage of *Uvs1*. The parental strains chosen were BALB/c which showed low susceptibility to UV-induced immunosuppression and C57BL/6 which showed high susceptibility. Offspring of the backcross, BALB/c female x (BALB/c x C57BL/6) F_1 [CB6F_1] male, showed equal segregation into parental phenotypes due to segregation of *Uvs1*. The action of *Uvs1* was isolated in this backcross since *Uvs3* was fixed as the BALB/c allele and, since *Uvs2* is recessive, the action of the C57BL/6 allele was not detectable. Backcross animals were tested twice at 4-7 month intervals for susceptibility to UV immunosuppression using a standard UV dose and determining the systemic effect on the contact hypersensitivity response *in vivo*. Trinitrochlorobenzene was used as contact sensitizer in the first test and oxazolone in the second test. The assignment of phenotype, i.e. high or low susceptibility to UV immunosuppression, was made on the basis of data for UV suppression derived with the same standard UV dose in the parental strains, 123 BALB/c females and 110 CB6F1 males. 73 backcross animals were selected with the extremes of phenotype based on parental values i.e. in each test the probability of misassignment of phenotype was < 0.05 for a probability of misassignment in both tests of <0.0025. DNA from these animals was subjected to a genome wide scan with 93 mapped markers (DMit series) detectable by PCR polymorphisms and spaced at 15-20cM. Data for nominal phenotype i.e. high or low susceptibility, together with the genotype data, was analyzed using the MapManager program. Linkage with a LOD score of 3.0 was found to 3 closely linked markers, D6Mit 54 and D6Mit389 at 48.2 cM and D6Mit287 at 49 cM 6 (Chromosome committee values).on chromosome 6. Using quantitative values for phenotype (% suppression) and the MapManagerQt program, linkage was demonstrated to the same 3 markers ($P= 0.0025$) and to D6Mit 327 (46.7cM, $P= 0.014$) and to D6Mit368 (55 cM, $P=0.048$). No significant linkage was found elsewhere. Establishing the location of *Uvs1* on chromosome 6 has eliminated a large number of potential genes as candidates for *Uvs1* including the major histocompatibility complex and most cytokine and cytokine receptor genes. We have recently demonstrated that dermal mast cell numbers are a critical determinant of susceptibility to UV-induced immunosuppression. Candidate genes on chromosome 6 which show mast cell related activity include the histamine H-1 receptor (*Hrh1*), interleukin 5ra (*Il5ra*), microphthalmia (*Mit*) and the tumor necrosis factor a receptor (*Tnfr1*). Alternatively, *Uvs1* may be a novel gene. Fine mapping of the *Uvs1* locus is anticipated to refine the limits of location of *Uvs1* and facilitate its identification.

D35. COMBINATORIAL GENOTYPES PRODUCE EXTREME INFLAMMATORY RESPONSE PHENOTYPES IN RI STRAINS.

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Multiple Organ Dysfunction Syndrome (MODS) is the leading cause of morbidity and mortality in surgical intensive care units. The sequential shutdown of lung, liver, kidney and gut is thought to result from an inappropriately regulated host inflammatory response to trauma and sepsis. There is currently no predictor for which patients will develop MODS, but genetic background may make an important contribution to susceptibility to this multifactorial disease. To map modifier genes, several inflammatory response phenotypes have been differentiated in A/J and C57BL/6J (B6) mice. An *in vitro* assay for mitogenesis in cultured splenic B-cells stimulated with *E-coli* endotoxin (LPS) consistently identified A/J cells as low responders and B6 as high responders. Reciprocal F_1's derived from a cross between A/J and B6 parents also tested as high responders in this assay, while the small number of backcross animals assayed yielded a wide range of responses. These results are consistent with the hypothesis that multiple non-imprinted autosomal genes contribute to this phenotype. Contributing genes were mapped by assaying 26 recombinant inbred (RI) strains of the AXB/BXA strain set. We identified six contributing *Mol* loci (*mo*difiers of *L*PS-response) that map to MMU 1, 7 (two different loci), 11, and 13 (two loci). Three of these loci map to regions known to confirm susceptibility to pathogens, including *Bcg*, *Leishmania*, *Legionella*, and *Salmonella*. The locus on MMU 1 falls in the same region as *Nramp1*; this localization has been independently confirmed and refined using C.D2VIL-6/Cr congenic mice. Three RI strains were hyperresponsive to LPS, showing mitogenesis levels 500-700% of the B6 (high) response, distinct from either parental strain. These strains are identical at the six *Mol* loci, with 4 A/J alleles and 2 B6 alleles. Two hyporesponsive strains were complementary with respect to A/J or B6 at all 6 loci. These findings provide a basis for further genetic refinement of the Mol loci and for the eventual identification of the responsible genes by positional cloning. Comparative mapping will allow us to localize the human homologs of these genes and to assess their contributions to the etiology of MODS.

D36. TWO LOCI ON CHROMOSOME 7 AND 14 INFLUENCE OSTEOSARCOMA SUSCEPTIBILITY IN C3H AND THE 102 MICE AFTER INCORPORATION OF 227THORIUM.

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The incidence of Bone Tumour in mice can be significantly increased by the injection of short-living, bone-seeking alpha-emitter such as 227Th. Although this is a general finding in all mice strains, marked differences in the latency-period suggest that genetic factors influence predisposition for osteosarcoma. Here we used an T-stock x (C3H x 102) F1-hybrid breeding system to investigate possible linkage of chromosomal region in the mouse genome with osteosarcoma susceptibility. Using a set of microsatellite-marker that cover the whole genome, we could exclude 95% of it from harbouring any susceptibility genes. Instead, two regions on chromosome 7 and 14, each narrowed down to approximately 15cM, exhibit significant linkage. In the region around *D7Mit229*, the C3H-allele conferred susceptibility (p=0.002), whereas at *D14Mit125* the allele from the 102-strain cosegregated with OS-susceptibility (p=0.016). Inheritance of the susceptibility-alleles in both region (i.e., haplotyp D7C3HD14102) resulted in an average OS-incidence of 73%, whereas inheritance of both resistance-alleles (i.e., haplotyp D7102D14C3H) yield an OS-incidence of only 13%. Interestingly, mice with the haplotypes representing the two parental inbred strains, i.e. D7C3HD14C3H and D7102D14102 showed an intermediate OS-incidence of 45% and 50%, resp. This suggests an additive interaction of two genes both influencing the occurrence of osteosarcoma.

Candidate genes in the linking regions for OS-predisposition are *Xrcc1*, *Bax* and *Fosb* (all in a 15cM region around *D7Mit229*) and *Rb1* (4cM downstream of *D14Mit125*).

D37. POSITIONAL CLONING OF THE MOUSE SKELETAL MUTATION, TAIL SHORT (*Ts*).

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A mouse mutation Tail-short (*Ts*) exhibits shortened kinky tail and numerous skeletal abnormalities including homeotic anteroposterior patterning problem along the axial skeleton. Ts gene was previously mapped to the teromeric region of chromosome 11. *Ts* is likely to be a mouse model for a human skeletal dysmorphology known as Meckel syndrome (MES;OMIM2400), since MES has phenotypes similar to Ts and has been mapped to human syntenic region, 17q21-24.

To elucidate the function of the *Ts* gene in mouse embryogenesis and verify whether it is a model for MES, we are trying to clone the gene by using the method of positional cloning. First, we employed a fine mapping of this gene based on a large scale intersubspecific backcross between the mutant stock TsJ/Le-Ts/+ and Japanese wild mouse-derived MSM strains. *Ts* gene was mapped to a 0.16cM region between two microsatellite markers, *D11Mit128* and *D11Mit256*. We screened mouse YAC and BAC libraries with the microsatellite markers tightly linked to the *Ts* locus and have obtained YAC and BAC clones. Further chromosome walking with the isolated clones allowed us to construct a complete BAC contig covering the *Ts* causative gene. This contig consists of 6 BAC clones, which spans a 250-300 kb DNA fragment. We have isolated several cDNA clones from the critical region by directed cDNA selection using the corresponding BAC clones to search for candidate genes for *Ts*. Characterization of these cDNA clones is now underway to identify the *Ts* causative gene.

D38. DYSTONIA AND DEFICIENCY OF SODIUM CHANNEL SCN8A: INFLUENCE OF A MODIFIER LOCUS ON MOUSE CHROMOSOME 3.

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Four mutant alleles of the voltage gated neuronal sodium channel *Scn8a* have been characterized (Meisler et al, Ann. Med. (Helsinki) 29:569). Null mutations result in progressive paralysis and are lethal at 3 to 4 weeks of age due to loss of motor neuron function. The hypomorphic medJ allele carries a 4 bp splice site deletion that results in incorrect splicing of 99% of transcripts and early termination of the protein (Kohrman et al, J. Biol. Chem. 271: 17576). When maintained on a C57BL/6J genetic background, the phenotype of homozygous medJ mice closely resembles that of the null mutants, except that survival is prolonged for 4 to 5 days. However, in the N4 generation of a backcross to strain C3H, most of the medJ homozygotes survived, suggesting the presence of an allele conferring resistance to the *Scn8a* deficiency. To probe the genetic basis of the resistant phenotype, we analyzed crosses between C57BL/6J-medJ and three inbred strains: C3H, DBA/2J and A/J. In all three crosses, two thirds of the medJ homozygotes in the F2 generation survived beyond 6 months of age. Since the distribution of survival time in the F2 is biphasic, homozygous medJ/medJ animals could be classified as susceptible (up to 1 month survival) or resistant (greater than 6 month survival). The phenotype of the long-lived medJ homozygotes includes severe muscle weakness that makes it difficult for the animals to support their weight. In addition, these animals have a dystonic movement disorder characterized by sustained abnormal limb postures and axial twisting.

A genome scan was carried out on pooled genomic DNA from 19 susceptible homozygotes and 20 resistant F2 homozygotes from the C3H cross. A skewed distribution was observed for alleles of locus *D3Mit6* (17.5 cM). Genotyping of 47 individual F2 animals enabled us to map the modifier locus to a 3 cM region of chromosome 3, between *D3Mit101* and *D3Mit214*. The same locus segregates in the crosses with strains DBA/2J and A/J, suggesting that C57BL/6J is unique in carrying the susceptible allele. The abundance of correctly and incorrectly spliced transcripts does not differ between susceptible and resistant mice in the C3H F2 generation; both have approximately 1% of normally spliced *Scn8a* transcripts. The function of the modifier locus in ameliorating the effects of *Scn8a* deficiency is of great interest for understanding sodium channel function in neurons. The modifier might influence subcellular localization, protein stabilization, or interaction of this sodium channel with membrane anchoring proteins.

D39. DIFFERENT MODE OF INHERITANCE IN DIABETES-RELATED PHENOTYPES IN SPONTANEOUSLY DIADETIC NSY MICE.

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Non-insulin dependent diabetes mellitus (NIDDM) is a major public health problem in western countries, leading to blindness, renal failure and early death. NIDDM have a strong genetic background. However, the major determinants of genetic susceptibility to NIDDM are still unknown The NSY (Nagoya-Shibata-Yasuda) mouse is an inbred strain of mice with spontaneous development of diabetes mellitus with moderate obesity (Ueda H et al. Diabetologia 1995). NSY mice spontaneously develop diabetes in an age-dependent manner. The cumulative incidence of diabetes is 98% in males and 31% in females at 48 weeks of age. Both impaired insulin secretion in response to glucose and insulin resistance contribute to the development of diabetes in NSY mice. In this study, two reciprocal crosses, female C3H x male NSY F1 (C3NF1) and female NSY x male C3H F1 (NC3F1) mice, were performed. The cumulative incidence of diabetes is 100% (25/25) in male C3NF1 mice and 97% (29/30) in male NC3F1 mice at 48 weeks of age, indicating that diabetes in NSY mice was transmitted to F1 hybrids in an autosomal dominant manner. Insulin resistance also showed an autosomal dominant mode of inheritance. In contrast, impaired insulin secretion inresponse to glucose showed an autosomal recessive mode of inheritance. BMI and fat accumulation showed a co-dominant mode of inheritance. These data indicated different mode of inheritance depending on diabetes-related phenotypes studied. Whole genome screening of susceptibility genes for NIDDM is now underway in breeding studies in crosses of NSY with control mice.

D40. GENETIC AND PHYSICAL MAPPING OF *Idd4* tHAT CONTROLS ONSET OF IDDM IN MICE.

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The development of IDDM in the NOD mouse is under the control of multiple insulin dependent diabetes (*Idd*) genes. One of them, *Idd4*, was mapped to mouse chromosome 11 and defined as a broad peak in a linkage analysis based on a cross between NOD and B10.H-2^{g7} strains (Todd J.A.et.al, 1991,1993). For fine mapping of the *Idd4* gene without any influence of other susceptibility genes, we have established a congenic strain for *Idd4* by introducing the chromosomal segment of the MSM strain, which spans from *Acrb* to *Mpo* on chromosome 11, into the NOD genetic background. The incidence of diabetes at early onset (~20wks) in female NOD/Shi.*Idd4*$^{msm/msm}$ mice was somewhat higher than that in NOD mice or NOD/Shi.*Idd4*$^{nod/msm}$ congenic mice. This result indicated that an *Idd4* allele from the non-diabetic MSM is more susceptible to early onset of diabetes than that of NOD mice. In this study, we produced a series of eight recombinant strains which carry various segments of the MSM-derived chromosome in the congenic region containing *Idd4*. By comparing the incidence of early onset diabetes among these recombinant strains, we mapped *Idd4* within a short segment less than approximately 0.8cM on chromosome 11. To construct a physical map across the *Idd4* region, YAC and BAC libraries were screened with the DNA markers on the region. Finally, a contig of the three YAC and twelve BAC clones was obtained, which completely covered the *Idd4* region.

D41. NEW QTLS THAT MODULATE EYE AND BRAIN DEVELOPMENT: COMPARISON OF RIs, F2s AND AN ADVANCED INTERCROSS.

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We have mapped 12 QTLs that have specific effects on the size of eyes, retinas, and brains of mice. Genome-wide probabilities for most QTLs are well under 0.05. All phenotype data were adjusted by multiple linear regression to minimize non-selective effects of age, body weight, sex, litter size, and parity. Weights of brain components (forebrain, hindbrain, cerebellum, olfactory bulbs, and hippocampus) were corrected to eliminate global effects of brain weight. Data for ~5000 animals belonging to more than 150 strains are now available online at http://mickey.utmem.edu.

Using the BXD recombinant inbred (RI) strains we succeeded in mapping one or more QTL for several traits, including: (i) brain weight (proximal Chr 11), (ii) eye weight (proximal Chr 5), and (iii) cerebellar weight (mid-distal 8). We have not yet succeeded in mapping QTLs that modulate the size of the hippocampus or olfactory bulbs, but we suspect our phenotype data are not sufficiently precise. BXD strains have proved to be efficient at detecting major-effect QTLs, and we are pursuing this productive RI analysis by adding AXB and BXA strains. QTLs that are mapped with RI strains have high precision compared to F2 crosses, and 2-LOD confidence intervals of RI QTLs is often under 8 cM.

F2 crosses have been used to detect additional QTLs, particularly those with effects that are too small to resolve with RI strains. For example, three new QTLs modulating the weight of cerebellum have been mapped using ~500 A/J by BXD5 F2 progeny on Chrs 2, 7, and 14. All QTLs mapped with F2s have large 2-LOD confidence intervals (15 to 40 cM). To gain the power of F2s but with the positional precision of RI strains we have generated an advanced intercross between B6 and D2 (G7 as of June 30, 1998). We have collected data on eye and brain weight for all progeny and are mapping QTLs in successive generations. In at least one instance, a QTL with strong statistics in BXDs and reciprocal F2 crosses has not been detected in advanced generations. This may be due to a disruption of key epistaticinteractions during the erosion of linkage disequilibrium.

D42. THE DROWNING MOUSE STRAIN DERIVED FROM TENASCIN DEFICIENT MICE.

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Tenascin (TN) is one of extracellular matrix proteins which is highly expressed in the tumors and developing tissues. In order to clarify the biological function of the TN , TN deficient mice have been created by homologous recombination. However, no apparent phenotypes were reported in earlier studies. During the course of breeding of TN deficient mice, we found some mice which exhibited abnormal behavior, such as head tossing, hyperlocomotion and drowning in the swimming pool. We further continued inbreeding and selecting the drowning mice for 13 generations, and have established TNKO strain. Our previous data from cross experiments indicated that drowning behavior is controlled by TN on chromosome 4 and another unknown gene. The purpose of this study is to search for gene loci which is involved in the drowning behavior of the TNKO mice. We produced F2 mice between TNKO and wild-derived mouse strain, MSM, and tested for swimming in the pool. Swimming behavior was recorded by video recorder, and drowned mice were recovered. Genomic DNA of drowned mice was extracted from liver or spleen and used for genotyping at tenascin gene locus and simple sequence length polymorphism loci on each chromosome by MIT markers. Up to now, we have found a significant linkage between the drowning trait and marker loci on chromosome 10. We will present the behavioral characterization of TNKO mice and the progress of genome scan to look for the gene loci involved in the drowning behavior.

E. Genetics of Development & Differentiation

Abstracts - Presentations

E1. FROM PHENOTYPE TO GENE: MAPPING OF MUTATIONS AND GENES IN THE ZEBRAFISH.

Robert Geisler, Gerd-Jörg Rauch, Stefanie Glaser, Jeremy Keenan, Heike Schauerte, Russell Ray, Silke Geiger-Rudolph, **Pascal Haffter.** *Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35/III, 72076 Tübingen, Germany.*

E2. AN H19 DELETION IN THE MOUSE RANDOMLY DESTABILIZES GENOMIC IMPRINTING OF THE COREGULATED H19 AND IGF2 GENES.

Michael R. Reed, *Arthur D. Riggs, Jeffrey R. Mann. Biology Dept., Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd, DUARTE, CA, 91010.*

E3. ENDOTHELIAL EMBRYONIC PROGENITOR CELLS AS A MODEL TO STUDY CARDIOVASCULAR DEVELOPMENT AND DISEASES.

A.K. Hatzopoulos [1,2], *J. Folkman*[3], *E. Vasile*[4], *G.K. Eiselen*[1], *J.M. Vidal*[1], *R.D. Rosenberg*[1]. [1]*MIT, Dept. of Biology, Cambridge MA 02139, USA;* [2]*GSF, Institute of Clinical Molecular Biology and Tumor Genetics, D-81377 Munich, Germany;* [3]*Children's Hospital, Boston, MA 02115, USA;* [4]*Beth Israel Hospital, Boston MA 02115, USA.*

E4. IDENTIFYING CANDIDATE GENES AND NEW PHENOTYPES FOR THE PROXIMAL ALBINO DELETIONS.

Mary E. Wines[1,2], *Charles DeRossi*[2], *Ying Shi*[2], *Bernadette C. Holdener*[2]. [1]*Dept. of Microbiology and Molecular Genetics;* [2]*Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-5215.*

E5. DISRUPTION OF THE *CBP* GENE AFFECTS FACIAL AND CARDIAC DEVELOPMENT, LONG-TERM MEMORY AND EARLY HAEMATOPOIESIS.

Yuichi Oike[*,‡], *Tadashi Kaname*[*], *Nobuyuki Takakura*[**], *Akira Hata*[§], *Takayoshi Mamiya*[II], *Toshitaka Nabeshima*[II], *Kimi Araki*[*], *Toshio Suda*[**], *Ken-ichi Yamamura*[*]. [*]*Department of Developmental Genetics,* [**]*Department of Cell Differentiation, Institute of Molecular Embryology and Genetics,* [‡]*Division of Cardiology, Kumamoto University School of Medicine, Kumamoto 862-0976, Japan.* [§]*Department of Public Health, Hokkaido University School of Medicine, Sapporo 060-8638, Japan.* [II]*Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466-8560, Japan.*

E6. MAINTENANCE OF EARLY CNS AND PNS STRUCTURES REQUIRES THE DELTA HOMOLOGUE Dll1.

Kurt Wünsch[§], *Achim Gossler*[*], *Martin Hrabé de Angelis*[§]. [§]*GSF, Institute of Mammalian Genetics, Neuherberg, Germany;* [*]*The Jackson Laboratory, Bar Harbor; ME; USA.*

E. Genetics of Development & Differentiation

E1. FROM PHENOTYPE TO GENE: MAPPING OF MUTATIONS AND GENES IN THE ZEBRAFISH.

Robert Geisler, Gerd-Jörg Rauch, Stefanie Glaser, Jeremy Keenan, Heike Schauerte, Russell Ray, Silke Geiger-Rudolph, **Pascal Haffter.** *Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35/III, 72076 Tübingen, Germany.*

Large-scale systematic mutagenesis screens have identified over 1000 mutations affecting genes with specific functions during early development, differentiation and organogenesis in the zebrafish. Genetic complementation analysis has assigned 894 of these mutations to 372 distinct genetic loci whereas for 269 mutations complementation still remains unresolved. Our aim is to place these 641 genetic loci on a map of simple sequence length polymorphisms (SSLPs). A genetic map of 2000 SSLP markers spanning the genome at an average spacing of 1.3cM has been generated by Knapik et al. The strategy used for mapping mutations involves a bulked segregant analysis, in which SSLPs are amplified from pools of 48 mutants and siblings, and separated on agarose gels. A difference in band intensity between the mutant and sibling pools indicates a potential linkage to the SSLP in question, which is confirmed and refined by genotyping single embryos. To date we have found a significant linkage to at least one marker for over 150 mutations. To complement these mapping efforts, we have generated a whole genome radiation hybrid map of the zebrafish genome consisting of 1000 SSLPs, STSs, cloned cDNAs and ESTs.

Our lab pursues different strategies to further analyze some of the mapped mutations, with a particular focus on those affecting midline signaling and left-right asymmetry:

(a) Matching with previously cloned candidate genes. Selected candidate genes are placed on the SSLP map through restriction fragment length polymorphisms (RFLPs). So far we have identified numerous linkages between mutations and candidate genes of which 4 were confirmed by the sequencing of mutant alleles.

(b) Positional cloning, which is currently feasible only for a few mutations due to the limited density of the map. We were able to identify markers closely linked to iguana, a gene involved in sonic hedgehog signaling at the midline and are performing a genomic walk towards this locus.

(c) Generation of additional closely linked markers by the AFLP technique, which is greatly facilitated by preexisting map information. We are currently following this approach for several mutations.

E2. AN H19 DELETION IN THE MOUSE RANDOMLY DESTABILIZES GENOMIC IMPRINTING OF THE COREGULATED H19 AND IGF2 GENES.

Michael R. Reed, *Arthur D. Riggs, Jeffrey R. Mann. Biology Dept., Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd, DUARTE, CA, 91010.*

Genomic imprinting is a form of epigenetic gene regulation which results in parental-specific monoallelic expression of a number of autosomal genes scattered throughout the mammalian genome. Cytosine methylation of DNA is required for maintenance of monoallelic expression of most characterized imprinted genes, however the germ line signals which establish these methylation patterns are unknown. *H19* and *Igf2* are two imprinted genes situated 90 kb apart on mouse distal chromosome 7. The two genes share regulatory elements and are expressed from opposite alleles (*H19* maternally, *Igf2* paternally). DNA methylation is intimately connected with monoallelic expression of both genes. In both cases the paternal allele is methylated, corresponding to repression of *H19* and expression of *Igf2*. Two regions located 2 and 5 kb upstream of *H19* are methylated in sperm but not oocytes and so are candidates for the germ line imprint. We have created a mutation at the *H19* locus which disrupts embryonic expression of *Igf2* when inherited maternally. Half of the embryos correctly express *Igf2* monoallelically while the other half express Igf2 biallelically. The deletion therefore destabilizes the establishment of *Igf2* repression on the maternal chromosome. When inherited paternally, the deletion has very little effect on the expression of *Igf2*. We present a model that proposes an oocyte-specific inhibitor of methylation at the *H19* locus as being responsible for establishing correct monoallelic expression of *H19* and *Igf2*.

E3. ENDOTHELIAL EMBRYONIC PROGENITOR CELLS AS A MODEL TO STUDY CARDIOVASCULAR DEVELOPMENT AND DISEASES.

A.K. Hatzopoulos [1,2], *J. Folkman*[3], *E. Vasile*[4], *G.K. Eiselen*[1], *J.M. Vidal*[1], *R.D. Rosenberg*[1]. [1]*MIT, Dept. of Biology, Cambridge MA 02139, USA;* [2]*GSF, Institute of Clinical Molecular Biology and Tumor Genetics, D-81377 Munich, Germany;* [3]*Children's Hospital, Boston, MA 02115, USA;* [4]*Beth Israel Hospital, Boston MA 02115, USA.*

The aim of this study is to utilize embryonic endothelial stem cells as an experimental system to isolate novel endothelial cell growth and differentiation protein factors, discover genes with critical functions in embryonic and adult vasculature, and explore the potential of endothelial progenitors as venues for gene therapy. Using a combination of appropriate stromal cell layers and growth conditions, we have isolated a population of round cells from mouse embryos at day 7.5 to 7.8 that display characteristic properties of endothelial progenitors. RNA analysis demonstrates that the isolated cells express endothelial specific genes like the angiopoietin-1 receptor tie-2 and the thrombin receptor thrombomodulin (TM) as well as general mesodermal markers like fgf-3. *In vitro* differentiation with retinoic acid and cAMP leads to a strong induction of the Vascular Endothelial Growth Factor (VEGF) receptor flk-1 and von Willebrand Factor (vWF). Change to the characteristic cobblestone endothelial morphology and tube formation occurs by placing the embryonic cells in Matrigel, a substrate that favors growth of endothelial cells. Injection of the mouse cells into chicken embryos, showed incorporation of the cells in the endocardium of the embryonic heart and in the brain vasculature. These data demonstrate that the isolated cells represent early embryonic progenitors of endothelium. In initial experiments, we have screened supernatants from 25 tumor cell lines. So far we have identified three tumor lines which secrete factors that affect growth and/or differentiation of the endothelial progenitor cells. We are currently characterizing the nature of the inducing factors.

The isolation of the embryonic endothelial progenitors is reproducible. The cells display unlimited stem-cell like growth in culture without phenotypic changes and they can be genetically manipulated by standard transfection techniques. These are the first embryonic endothelial cell progenitors isolated in mammals and thus provide a unique and novel system to study vascular development and endothelial cell function in terms of cellular interactions, inducing signals and transcriptional regulation.

E4. IDENTIFYING CANDIDATE GENES AND NEW PHENOTYPES FOR THE PROXIMAL ALBINO DELETIONS.

Mary E. Wines[1,2], *Charles DeRossi[2], Ying Shi[2], Bernadette C. Holdener[2]. [1]Dept. of Microbiology and Molecular Genetics; [2]Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-5215.*

The x-ray induced albino deletions of mouse chromosome 7 identify at least six functional intervals by complementation analysis. Several of these deletions are being utilized in our lab to study mesoderm induction and palate formation. Embryos homozygous for deletions that remove the mesoderm development (mesd) functional region lack mesoderm. Adjacent to mesd , we have identified a region functioning in palate morphogenesis. To aid in characterization of these essential functional regions, we have constructed a 1.5 Mb physical map using BACs and YACs spanning critical deletion breakpoints. We estimate that the mesd interval is a maximum of 800 kb and demonstrate that the palate region is 350 kb.

Mesd embryos do not express posterior primitive streak markers. To determine whether an A-P axis is established in mutants, we are characterizing anterior marker expression. In addition to primitive streak defects, mesd mutant cells fail to form mesoderm when grafted in vivo. Using chimera analysis (wt ES cells ---> mesd embryos) we demonstrate that mesd is also required in visceral endoderm for mesoderm induction. Genomic sequencing of the mouse BACs followed by BLAST alignment has revealed high identity to a human trabecular bone cell EST, providing a likely candidate for mesd. Candidate transcripts are present at high levels at 7.0 dpc, decrease throughout gestation, and are detectable at low levels in brain and testes in the adult. Embryonic in situ analysis in parallel with ES cell rescue will evaluate the gene's candidacy as a mesoderm inducer.

Sixty-five percent of embryos homozygous for the deletion encompassing the palate region have cleft palates. This finding is consistent with recent linkage studies mapping a teratogen-inducible cleft palate suscept-bility locus to 43 cM on mouse chromosome 7 near the palate interval (Diehl et al, PNAS 94(10): 5231-5236). Within this region we have identified the Aryl hydrocarbon receptor nuclear translocator-2 (*Arnt2*) gene which covers 140 kb (40%) of the palate interval. Previous work has shown that dioxin, a known teratogen and ligand of the Aryl hydrocarbon receptor (AhR), induces palate clefting (Abbott et al., Toxicol. Appl. Pharmacol. 128(1): 138-150). Since AhR is a dimerization partner for *Arnt2*, this finding supports *Arnt2* as a likely candidate for the palate morphogenesis regulator.

E5. DISRUPTION OF THE *CBP* GENE AFFECTS FACIAL AND CARDIAC DEVELOPMENT, LONG-TERM MEMORY AND EARLY HAEMATOPOIESIS.

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In the present study, we generated *CBP* disruption mice using gene-trapping strategy. Heterozygous CBP deficient mice, which had truncated CBP protein (residues1-1084) containing CREB binding domain (residues 462-661) showed clinical features of Rubinstein-Taybi syndrome (RTS), such as growth retardation (100%), retarded osseous maturation (100%), hypoplastic maxilla with narrow palate (100%), cardiac anomalies (15%), and skeletal abnormalities (7%). Truncated CBP is considered to act during development as a dominant negative inhibitor leading the phenotype of RTS in mice. Our studies with step-through type passive avoidance test and with water finding test showed that mice were deficient in long-term memory (LTM). In contrast, short-term memory, studied with Y-maze test, was normal. Together with the findings of CREB deficient mice, these results implicate the involvement of CREB-dependent transcription in mammalian LTM. On the other hand, the homozygous mutants died in utero between 9.5 and 10.5 dpc (days postcoitum) with abnormalities in the development of the early hematopoietic system.

E6. MAINTENANCE OF EARLY CNS AND PNS STRUCTURES REQUIRES THE DELTA HOMOLOGUE Dll1.

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The *Delta* (*Dl*) and *Notch* (*N*) genes of *Drosophila* encode transmembrane proteins that interact in a ligand-receptor-like manner and are involved in cell-to-cell communication processes regulating the determination of various cell fates during embryonic and larval development. In the mouse, the Delta homologue *Dll1* (*Delta-like gene 1*) and *Notch1* show a strikingly similar expression pattern in the paraxial mesoderm and nervous system during early postimplantation development suggesting that cells in these tissues can communicate by interaction of Dll1 and Notch1 proteins

Dll1 null embryos show impairment in somitic and neuronal development. The *Dll1*$^{-/-}$ mutation is embryonically lethal approximately at day 11.5 post coitus (pc). Within the CNS *Dll1* k.o. embryos do not show major morphological changes before day 9.5 pc. However, at day 10.5 pc distinct regions in the CNS appear hyperplastic and interluminal bleeding is visible. Morphological analysis of the CNS reveals a dramatic reduction in number of cells within the ventricular layer and the intermediate zone seems disorganized. Ruptures within the ventral half of the neuro-epithelium and associated bleeding into the ventricular lumen may be the cause of early embryonic lethality.

Genes involved in the Delta-Notch-signal-transduction pathway show altered gene expression in *Dll*$^{1-/-}$ embryos. At day 9.5 pc expression of *Mash-1* is upregulated in *Dll1* null embryos and a downregulation of *Hes-5* transcripts can be observed within the dorsal neural tube. At day 10.5 pc *Hes-5* transcripts are lost from the dorsal tip of the neural tube and *Mash-1* expression seems to be farther upregulated in this region.

Other proneural (e.g. *Math4a, NeuroD*) and neuronal (e.g. *Neurofilament, Islet-1*) genes also show altered expression within the developing CNS and PNS of *Dll1* null embryos. Also expression of *Dll3*, another potential Notch ligand is modified in *Dll*$^{1-/-}$ embryos.

E. Genetics of Development & Differentiation

Abstracts - Posters

E7. A SYSTEMATIC MOLECULAR GENETIC APPROACH TO STUDY MAMMALIAN GERM LINE DEVELOPMENT.

Kuniya Abe[1], *Kanae Mitsunaga*[1], *Marija J. Grahovac, Meng K. Lim*[2], *Grant R. MacGregor*[3], *Minoru S. H. Ko*[2]. [1]*Inst. Mol. Emb. & Genet., Kumamoto Univ. School of Med., Kumamoto 862, Japan.* [2]*Center for Molecular Medicine and Genetics, Wayne State Univ. School of Med., Detroit, MI 48202, USA.* [3]*Center for Molecular Medicine, Emory Univ. School of Med., Atlanta, GA 30322, USA.*

E8. THE DEVELOPMENTAL ROLE OF UNCONVENTIONAL MYOSIN VI AND ITS ASSOCIATION WITH DEAFNESS.

Karen B. Avraham[1], *Orit Hadad*[1], *Tim Self*[2], *Tama Sobe*[1], *Karen P Steel*[2]. [1]*Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel;* [2]*MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, UK.*

E9. *Math5* ENCODES A BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR SPECIFICALLY EXPRESSED IN THE DEVELOPING MOUSE RETINA.

Nadean L. Brown[1], *Shami Kanekar*[2], *Monica L. Vetter*[2], *Priscilla K. Tucker*[3], *Debi L. Gemza*[1], *Tom M. Glaser*[1]. [1]*Howard Hughes Medical Institute and Departments of Human Genetics and Internal Medicine, University of Michigan;* [2]*Department of Neurobiology and Anatomy, University of Utah;* [3]*Museum of Zoology and Department of Biology, University of Michigan.*

E10. TCF-4 IS EXPRESSED IN DISTINCT REGIONS OF THE EMBRYONIC BRAIN AND LIMBS.

Eun Ah Cho, *Gregory R. Dressler. Department of Pathology and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109.*

E11. GENES IN THE SEX DETERMINATION/DIFFERENTIATION PATHWAY OF MICE.

William Crain, *Jill O'Moore. McLaughlin Research Institute, Great Falls, MT59405.*

E12. DEVELOPMENTAL ABNORMALITIES IN DOUBLEFOOT HOMOZYGOTES: ELABORATION OF A NEW GENE INVOLVED IN HEDGEHOG SIGNALLING.

Christopher Hayes[*$], *Andy Haynes*[$], *Mary Lyon*[**], *Paul Denny*[$], *Gillian Morriss-Kay*[*], *Steve Brown*[**$]. [*]*Department of Human Anatomy, University of Oxford, South Parks Road Oxford, OX1 3QX;* [**]*MRC Mammalian Genetics Unit;* [$]*MRC Mouse Genome Centre, Harwell, Didcot, Oxon., OX11 ORD.*

E13. MOLECULAR GENETIC ANALYSIS OF THE MOUSE MUTATION tail kinks.

Urlich Heinzmann[1], *Merve Olowson*[2], *Andreas Püschel* [3], *Heinz Höfler*[1], *Rudi Balling*[2], *Kenji Imai*[2]. *GSF-National Research Center for Environment and Health,* [1]*Institute of Pathology;* [2]*Institute of Mammalian Genetics2, D-85764 Neuherberg, Germany;* [3]*Molekulare Neurogenetik, Abteilung und Neurochemie, Max-Planck-Institut für Hirnforschung, Deutschordenstrasse 46, D-60528 Frankfurt am Main, Germany.*

E14. POLYMORPHISM AT TAIL-SHORT (*Ts*) LOCUS AMONG STANDARD INBRED STRAINS AFFECTS THE VIABILITY OF *TS* HETEROZYGOTES.

Junko Ishijima[1], *Hiroshi Yasui*[2], *Masae Morishima*[3], *Toshihiko Shiroishi*[1]. [1]*Mammalian Genetics Laboratory, National Institute of Genetics,Yata-1111 Mishima, Shizuoka-ken 411, Japan;* [2]*Department of Anatomy and Developmental Biology, Tokyo Women's Medical College, Tokyo 162, Japan;* [3]*Research Division, Heart Instiitute of Japan, Tokyo Women's Medical College, Tokyo 162, Japan.*

E15. DOSAGE-DEPENDENT EFFECTS OF *qkI*, ENCODING A KH-CONTAINING RNA BINDING PROTEIN, ON INITIATION OF MYELINATION IN CNS AND EMBRYONIC DEVELOPMENT.

T. Kondo[1], *T. Kaname*[1], *Kanae Mitsunaga*[1], *Sun Feng*[1], *T. Ebersole*[2], *K. Artzt*[2], *K.Yamamura*[1], *K. Abe*[1]. [1]*Inst. Mol. Emb. Genet., Kumamoto Univ. Sch. Med.;* [2]*Dept. Zool., Univ. Texas at Austin.*

E16. DOWNREGULATION OF SONIC HEDGEHOG (*Shh*) AND PATCHED (*Ptc*) EXPRESSION IN AN X-RAY INDUCED MOUSE MUTANT EXHIBITING LIMB AND FACIAL ABNORMALITIES.

Ottheinz Krebs[1], *Arne Luz*[2], *Heidi Alt*[1], *Jack Favor*[3], *Eckhard Wolf*[1]. *Institute of Molecular Animal Breeding, Gene Center, D-81377 Munich1, Institute of Pathology2; Institute of Mammalian Genetics3, GSF, D-85764 Neuherberg, Germany.*

E17. MORPHOLOGICAL AND DEVELOPMENTAL STUDY ON A NEW MUTANT KNOTTY-TAIL (*knt/knt*) MOUSE.

Tetsuro Matsuura[1], *Isao Narama*[1], *Kiyokazu Ozaki*[1], *Hiroo Nakajima*[2], *Masato Uehara*[3]. [1]*Research Institute of Drug Safety, Setsunan University, Osaka, Japan;* [2]*Department of Radiation Biology, Faculty of Medicine, Osaka University, Osaka, Japan;* [3]*Department of Veterinary Anatomy, Faculty of Agriculture, Tottori University, Tottori, Japan.*

E18. **MULTIMERIC DNA BINDING COMPLEXES INVOLVING THE Meis1, Pbx AND Hox HOMEOPROTEIN FAMILIES IN MURINE MYELOID LEUKEMIA.**

Jeffry C. Montgomery, *Scott Steelman, Arthur M. Buchberg. Kimmel Cancer Center, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, 19107.*

E19. **ANALYSIS OF THE qk^{k2} EMBRYO LETHAL ALLELE AND qk^{e5}, A NEW ENU INDUCED ALLELE OF QUAKING: THE DISCOVERY OF THE qk^{k2} MOLECULAR DEFECT AND A SEMIDOMINANT SEIZURE SUSCEPTIBILITY PHENOTYPE.**

J.K. Noveroske[1], *D.A. Carpenter*[2], *M.J. Justice*[3]. [1]*Department of Zoology, University of Tennessee, Knoxville, TN 37926;* [2]*Mammalian Genetics Section, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-8080;* [3]*Dept. of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.*

E20. **DEVELOPMENTAL EXPRESSION OF JAGGED1 IN THE MAMMALIAN HEART: IMPLICATIONS FOR CARDIAC DISEASE IN ALAGILLE SYNDROME.**

KM Loomes[1], *NB Spinner*[2], *DA Piccoli*[1], *HS Baldwin*[3], **RJ Oakey**[2]. *Divisions of Gastroenterology*[1], *Human Genetics*[2], *Cardiology*[3], *Children's Hospital of Phila., Univ. of PA School of Medicine, Philadelphia, PA.*

E21. **A COMMUNITY EFFECT IS RESPONSIBLE FOR A VARIEGATED COAT COLOR PHENOTYPE IN MICE.**

Geneviève Aubin-Houzelstein, **Jean-Jacques Panthier.** *URA INRA de Génétique Moléculaire, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général-de-Gaulle, Maisons-Alfort 94704 cedex, France.*

E22. **THE MOUSE AND HUMAN HOMOLOGS OF *Drosophila melanogaster neuralized* GENE ARE EXPRESSED DURING LIMB DEVELOPMENT.**

Elias K. Pavlopoulos[1,2], *Panagiotis Prinos*[3], *Maria Kokkinaki*[1,2], *Caroline Dealy*[4], *Elise Rose*[3], *Robert Kosher*[4], *Michael Kilpatrick*[3], *Nicholas K. Moschonas*[1,2], *Petros Tsipouras*[3]. *Department of* [1]*Biology, University of Crete;* [2]*Institute of Molecular Biology and Biotechnology, FORTH-GR, Heraklion, GR-714 09, Greece; Departments of* [3]*Pediatrics, 4 Anatomy, University of Connecticut Health Center, Farmington, CT 06030, USA.*

E23. **POSSIBLE INVOLVEMENT OF Mid1 IN THE PATCHY FUR (Paf) MUTANT.**

Nandita A. Quaderi, *Silvia Messali, Silvia Cainarca, Andrea Ballabio, Germana Meroni, Elena Rugarli. Telethon Institute of Genetics and Medicine (TIGEM), 58 via Olgettina , Milan, Italy.*

E24. **CHARACTERISATION OF X-LINKED DEVELOPMENTAL MUTANTS WITH CRANIOFACIAL ABNORMALITIES.**

Vivienne Reed, *Emmanuelle Gormally, Walter Masson, Terry Hacker, Yvonne Boyd. MRC Mammalian Genetics Unit, Harwell, Oxfordshire, England.*

E25. **EXPRESSION OF *Msx1* IN DERMOMYOTOME AND ITS DERIVATIVES.**

D. Houzelstein, Y. Chéraud, G. Auda-Boucher*, J. Fontaine-Pérus*, M. Buckingham ,* **B. Robert.** *Dept of Molecular Biology, Institut Pasteur, 75724 Paris Cedex15, France; *CNRS URA 1340, Faculté des Sciences et Techniques, 44000 Nantes, France.*

E26. **GENETIC ANALYSIS AND PHYSICAL MAPPING OF POLYDUCTYLOUS MOUSE MUTATION, HEMIMELIC EXTRA TOES (*Hx*).**

T. Sagai, *H. Masuya, T. Shiroishi. Mammalian Genetics Laboratory, National Institute of Genetics, Yata 1111, Mishima Shizuoka-ken 411-8540, Japan.*

E27. **AN ALLELIC SERIES INDUCED BY ENU AT *axis* REVEALS MULTIPLE FUNCTIONS DURING DEVELOPMENT.**

S.E. Thomas[1], *A.P. Davis*[2], *D.A. Carpenter*[2], *M.J. Justice*[1]. [1]*Dept. of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030;* [2]*Mammalian Genetics Section, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-8080.*

E28. **GENOME ANALYSIS OF THE MURINE *Pax1* DELETION MUTANT $Pax1^{Un\text{-}s}$ AND SEARCH FOR ADDITIONAL GENES INVOLVED IN THE $Pax1^{Un\text{-}s}$ MUTATION.**

Bettina Wilm, *Rudi Balling, Ken-ichi Yamamura*, Kuniya Abe*, Kenji Imai. GSF-National Research Center for Environment and Health, Institute of Mammalian Genetics, 85764 Neuherberg, Germany; *Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan.*

E7. A SYSTEMATIC MOLECULAR GENETIC APPROACH TO STUDY MAMMALIAN GERM LINE DEVELOPMENT.

Kuniya Abe[1], *Kanae Mitsunaga*[1], *Marija J. Grahovac, Meng K. Lim*[2], *Grant R. MacGregor*[3], *Minoru S. H. Ko*[2]. [1]*Inst. Mol. Emb. & Genet., Kumamoto Univ. School of Med., Kumamoto 862, Japan.* [2]*Center for Molecular Medicine and Genetics, Wayne State Univ. School of Med., Detroit, MI 48202, USA.* [3]*Center for Molecular Medicine, Emory Univ. School of Med., Atlanta, GA 30322, USA.*

Development of the germline is essential to the survival of a species. The primordial germ cells (PGCs) formed during mammalian embryogenesis ultimately give rise, in the male, to spermatogonia, the only self-renewing cell type in an adult capable of making a genetic contribution to the next generation. Obviously, contemporary mammalian germ cell research is of importance to both basic and applied biological science. However, despite this fact, our knowledge of the molecular basis for germline development during mammalian embryogenesis is relatively poor . Previous attempts to study gene expression in mouse PGC have been confounded by the difficulty inherent in obtaining both sufficient quantities and purity of PGCs. We have overcome this problem using a novel combination of molecular and transgenic approaches. A line of mice has been generated in which the cells of the germ lineage express the lacZ reporter gene. PGCs were purified from this TNAP$^{b\text{-}geo}$ embryo using FACS sorting of β-gal expressing cells. Analysis of the sorted cells by alkaline phosphatase staining and immuno-cytochemistry with anti-C-KIT antibody demonstrated that highly (98%) purified PGCs can be isolated using this method from various developmental stages (Abe et al. Develop. Biol. 180, 468-472, 1996). The ability to isolate highly pure populations of PGCs enables many interesting experiments to delineate molecular and cellular characteristics of PGC. For example, methylation status of the PGC genome or telomerase activity in PGC can be studied using purified PGC samples. We have amplified whole cDNA population derived from purified PGCs using PCR-based techniques (K. Abe Mamm. Genome 2, 252-259, 1992), and constructed cDNA libraries. These cDNAs can be used to test the expression of known genes in PGC, or to monitor gene expression occurring in PGCs during germ line development. Currently, 4000 clones were picked up from e13.5 male and female PGC libraries, and these clones were subjected to DNA sequencing analysis.

Approximately 2000 informative cDNA sequences were obtained and analyzed by BLASTN search. About 50% of the sequences matched to known genes, 25% showed similarity to known genes, and the remaining, 25% seemed to be novel. Also, we found cDNA clones specifically appeared in either male or female library.

These libraries and the derived information will constitute an important resource for future functional studies to understand the biological process of germ cell development.

E8. THE DEVELOPMENTAL ROLE OF UNCONVENTIONAL MYOSIN VI AND ITS ASSOCIATION WITH DEAFNESS.

Karen B. Avraham[1], *Orit Hadad*[1], *Tim Self*[2], *Tama Sobe*[1], *Karen P Steel*[2]. [1]*Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel;* [2]*MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, UK.*

The inner ear is a complex structure, requiring a repertoire of genes turning on and off during development. The vertebrate inner ear is derived from the otic placode, a region of embryonic ectoderm that forms the otic vesicle early in development. The epithelium of the otic vesicle eventually gives rise to the sensory hair cells, which act as the primary receptor cells in the inner ear. The formation and structure of the hair cells is being studied in the Snell's waltzer mouse. This mouse deafness locus is encoded by an unconventional myosin, myosin VI (*Myo6*). Despite being broadly expressed in the mouse, the only consequence of genetic inactivation of myosin VI in mice is deafness and vestibular dysfunction, suggesting a specific role for myosin VI in the cells of the inner ear. Two Snell's waltzer alleles have been identified: *sv*, a spontaneous mutation, and *se^{sv}*, a radiation-induced mutation. The *sv* allele contains an intragenic deletion in *Myo6*, leading to a complete loss of this protein in all tissues; sesv exhibits a severe reduction in the levels of myosin VI expression. The effects of both mutations on cochlear hair cell development using scanning electron microscopy have revealed disorganization of the precise arrangement of stereocilia. In the whole embryo, *Myo6* is not yet expressed at embryonic day 7, but is expressed by embryonic day 11. During development, highest levels of expression are seen in the kidney, intestine and inner ear, all structures that contain epithelial cells.

E9. *Math5* ENCODES A BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR SPECIFICALLY EXPRESSED IN THE DEVELOPING MOUSE RETINA.

Nadean L. Brown[1], *Shami Kanekar*[2], *Monica L. Vetter*[2], *Priscilla K. Tucker*[3], *Debi L. Gemza*[1], *Tom M. Glaser*[1]. [1]*Howard Hughes Medical Institute and Departments of Human Genetics and Internal Medicine, University of Michigan;* [2]*Department of Neurobiology and Anatomy, University of Utah;* [3]*Museum of Zoology and Department of Biology, University of Michigan.*

We have identified and mapped *Math5*, a mouse basic helix-loop-helix (bHLH) family member that is very closely related to the *Drosophila atonal* and *Xenopus Xath5* genes and is specifically expressed in the developing eye. *Math5* retinal expression commences just prior to differentiation of the first neurons and persists within the progenitor cells until after birth. To position *Math5* in a retinal developmental hierarchy, we compared *Math5* expression, in wild type and *Pax6*-deficient (*Sey*) embryos, to *Hes1*, a bHLH gene that is also expressed at early stages of murine retinal neurogenesis. *Math5* expression is down-regulated in heterozygous (*Sey/+*) embryos and abolished in homozygous (*Sey/Sey*) mutant eye rudiments. Conversely, *Hes1* expression is upregulated by loss of *Pax6* in a similar dose-dependent manner. These results link *Pax6* to the process of retinal neurogenesis, and provide the first molecular correlate for the dosage-sensitivity of the *Pax6* phenotype. During retinogenesis, *Math5* is expressed significantly before *Mash1*. To test whether these genes influence the fates of distinct classes of retinal neurons, we ectopically expressed *Math5* and *Mash1* in *Xenopus* retinal progenitors. Unexpectedly, lipofection of either mouse gene into the frog retina produced an identical phenotype, an increase in differentiated bipolar cells at the expense of Müller glial cells. Directed expression of *Math5*, but not *Xath5*, in Xenopus blastomeres produced a novel retinal overgrowth phenotype. We propose that *Math5* acts as a proneural gene to determine the fate of particular retinal neurons, but has properties different from its most related vertebrate family member, *Xath5*.

E10. TCF-4 IS EXPRESSED IN DISTINCT REGIONS OF THE EMBRYONIC BRAIN AND LIMBS.

Eun Ah Cho, *Gregory R. Dressler. Department of Pathology and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109.*

The Tcf family of transcription factors, in association with β-catenin, mediate Wnt signaling by transactivating downstream target genes. Given the function of *wnt* genes in neural development and organogenesis, Tcf transcription factors must be integral to the development of many embryonic tissues. In fact, the role of Tcf genes in axis formation in *Xenopus* and in segment polarity in *Drosophila* is well established. We have identified two isoforms of the mouse *Tcf-4* gene, *4B* and *4E*. Cells transfected with Tcf-4 showed nuclear localization of b-catenin. Although Tcf-4 RNA was widely distributed throughout embryogenesis, high levels of Tcf-4 expression were particularly evident in the developing brain and limb buds. In extended streak stage embryos (E7.5), Tcf-4 expression was detected in anterior endoderm. E8.5 embryos had Tcf-4 expression in rostral end and also in alternating rhombomeres of the hindbrain. By E9.5 and thereafter, expression in the hindbrain disappeared and strong expression was detected in the diencephalon. Strikingly, Tcf-4 expression in the forebrain was undetected in *Small eye* mutant embryos indicating that *Pax-6* is required for Tcf-4 expression in the forebrain. In developing limbs, *Tcf-4* is readily detected starting at E10.5 and is limited to mesenchymal cells surrounding the areas of chondrofication. These data indicate a function for *Tcf-4* in neural and limb development, two tissues where Wnt signaling plays an essential role.

E11. GENES IN THE SEX DETERMINATION/DIFFERENTIATION PATHWAY OF MICE.

William Crain, *Jill O'Moore. McLaughlin Research Institute, Great Falls, MT59405.*

We are studying the genetic basis for sex determination and differentiation in mice. The sex of mammals is established at fertilization by whether or not the egg is fertilized by a Y-chromosome-bearing or an X-chromosome-bearing sperm. However, the morphological events that distinguish males and females do not begin to occur until about 11.5 days post coitum (d.p.c.) in mice. Until this time the cells of the urogenital ridges of both sexes are indistinguishable and have the potential to follow either of two normal, but distinct, differentiation pathways leading to the formation of either ovaries or testes. One gene on the Y chromosome, *Sry*, is activated in these cells at this time and initiates the pathway for testis differentiation. In its absence the genital ridges become ovaries. Although *Sry* was discovered in 1990 information about other genes in these alternate pathways is still quite limited. We are using differential display to search for other genes that are active exclusively during early stages of differentiation of the testes or ovaries. We are comparing the mRNA profiles from 11.5 and 12.5 d.p.c. genital ridges of male (XY), female (XX) and sex reversed XY fetuses (from the mouse strain C57l3L/6jEi-Y^{pos} developed by E. Eicher). So far, we have examined the expression of about 2000 mRNAs and have identified six with sex-specific expression: three that are present in all XY but not in XX fetuses, two that are only in females (XX) and one that is expressed in XY males but not XY sex reversed fetuses. One female-specific RNA is from the *Xist* gene which is directly involved in inactivation of one of the X chromosomes in females, but probably not directly in sex determination. One of the male-specific RNAs encodes the male-specific HY antigen and is thus also probably not involved directly in sex determination. The other four mRNAs are likely to be important in the early stages of sex determination/differentiation. One of these RNAs is from a gene on chromosome 8 encoding precerebellin, a protein that is also expressed in the Purkinje cells of the cerebellum. Using whole mount in situ hybridization we have shown that this RNA is present in the cords of the developing testis at 12.5 d.p.c., and not in the mesonephros. Currently, we are verifying the expression patterns of the other three, mapping their genes and searching for additional sex specific RNAs.

E12. DEVELOPMENTAL ABNORMALITIES IN DOUBLEFOOT HOMOZYGOTES: ELABORATION OF A NEW GENE INVOLVED IN HEDGEHOG SIGNALLING.

Christopher Hayes[*$], *Andy Haynes*[$], *Mary Lyon*[**], *Paul Denny*[$], *Gillian Morriss-Kay*[*], *Steve Brown*[**$]. [*]*Department of Human Anatomy, University of Oxford, South Parks Road Oxford, OX1 3QX;* [**]*MRC Mammalian Genetics Unit;* [$]*MRC Mouse Genome Centre, Harwell, Didcot, Oxon., OX11 ORD.*

The mouse mutant Doublefoot (dbf) exhibits preaxial polydactyly of all four limbs in association with craniofacial defects. These defects are due to the aberrant and ectopic activation of Hedgehog (Hh) signalling, due to the Dbf mutation. Here we describe additional developmental abnormalities in homozygous Dbf embryos, which are not recovered beyond the fifteenth day of gestation.

Dbf/Dbf embryos display defects not seen in heterozygous embryos, in particular abnormalities of branching morphogenesis of several organ systems where Hh signalling is known to be important, including the respiratory system, pancreas and kidneys. Mutations in characterised elements of the Hh signalling pathway have been implicated in the genesis of basal cell carcinomas (BCC) a common human cancer of the skin. The skin of adult Dbf heterozygotes display hyperproliferative defects and show increased density of hair follicles per unit area of skin consistent with a possible oncogenic role for Dbf.

We have begun the positional cloning of the doublefoot mutation. A large interspecific backcross of 681 progeny have been typed for markers in the vicinity of the doublefoot mutation on chromosome 1 and the doublefoot locus narrowed to a region of 0.9cM. We will report progress on the physical mapping of the doublefoot nonrecombinant region and the identification of candidate genes.

E13. MOLECULAR GENETIC ANALYSIS OF THE MOUSE MUTATION tail kinks.

Urlich Heinzmann[1], *Merve Olowson*[2], *Andreas Püschel* [3], *Heinz Höfler*[1], *Rudi Balling*[2], *Kenji Imai*[2]. *GSF-National Research Center for Environment and Health,* [1]*Institute of Pathology;* [2]*Institute of Mammalian Genetics2, D-85764 Neuherberg, Germany;* [3]*Molekulare Neurogenetik, Abteilung und Neurochemie, Max-Planck-Institut für Hirnforschung, Deutschordenstrasse 46, D-60528 Frankfurt am Main, Germany.*

The metameric structure of somites and the anterior-posterior (A-P) polarity within each somite have a fundamental influence on the segmental organization of the peripheral nervous system (PNS). We report a disturbance in the A-P patterning of somites in the mouse mutant tail kinks (tk). Affected tk animals show a characteristic malformation in the ventral part of the neural arches, the pedicles: they are completely lost or remain hypoplastic. Associating with this skeletal abnormality, we found a striking abnormality in the segmental organization of PNS with extensive fusions of the dorsal root ganglia (DRG). These skeletal and PNS phenotypes together reflect malformations experimentally produced in chick embryos where multiple anterior-half somites are grafted. Accordingly, we could show that somite marker genes including semaphorin D, Paraxis, and Pax9, which are normally expressed in the posterior half of somites, are strongly down-regulated in tk embryos before histological abnormalities become apparent as reduced cellular condensation in the sclerotome cells.

The tk mutation arose spontaneously and has been mapped to chromosome 9. tk has been considered to be defective solely in sclerotome differentiation in earlier studies, and the structural abnormalities in PNS have been overlooked. Our observation strongly suggests that the disturbance in the A-P patterning of somites is the primary defect in tk, and that this defect results in the malformations of the vertebral column and in disturbed segmental organization of PNS.

E14. POLYMORPHISM AT TAIL-SHORT (*Ts*) LOCUS AMONG STANDARD INBRED STRAINS AFFECTS THE VIABILITY OF *TS* HETEROZYGOTES.

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Tail-short (*Ts*) heterozygotes have variable types of malformation, including kinky tail, vertebral homeotic transformations, developmental retardation and neural tube defects. The expression of the phenotype depends on the mouse strain to which the mutant is crossed. The variation in the *Ts* phenotypes observed in crosses with different inbred strains ranges from viable to dominant full lethal. Detailed linkage analysis indicated that a single chromosomal region, genetically inseparable from the *Ts* locus, is responsible for the difference. This result suggests that there exists polymorphism at *Ts* locus among inbred strains, and the different manners of the interactions between the *Ts* mutant gene and polymorphic allele of the inbred strain may affect the viability of the *Ts* heterozygotes. Furthermore, we investigated the phenotype of the *Ts* mutation in crosses with the two groups of strains, which give viable and lethal *Ts* embryos respectively. *Ts* heterozygous embryos derived from the lethal cross exhibited more severe defects than those from the viable cross. Morphological anomalies of the *Ts* heterozygotes in the lethal cross were observed in as early as neural plate stage. The embryonic region was poorly developed compared with the extraembryonic region due to formation of excess mesoderm in the extraembryonic region. In the later developmental stage, the umbilical vein does not develop properly, which may be the cause of the lethality of the *Ts* heterozygotes around 12.5 dpc.

E15. DOSAGE-DEPENDENT EFFECTS OF *qkI*, ENCODING A KH-CONTAINING RNA BINDING PROTEIN, ON INITIATION OF MYELINATION IN CNS AND EMBRYONIC DEVELOPMENT.

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Quaking (*qk*) or *quaking viable* (qk^v) is a classical, recessive neurological mutation, showing rapid tremors when moving[1]. Recently, a candidate gene for this mutation, *qkI*, has been isolated in the process of positional cloning. *qkI* encodes a novel KH-containing RNA binding protein. In order to examine the relationship of the *qkI* with the *qk* mutation, and to understand the biological role of this gene, we conducted a series of experiments. Genomic region containing the *qk* locus has been isolated and assembled as YAC/BAC contigs. Genomic organization of the *qkI*, which gives rise to five alternative transcripts encoding four different products, was precisely determined. Based on such structural information, we constructed gene targeting vectors and a BAC construct for transgenic rescue experiment. A null, targeted allele, qkI^0, does not complement the original *qkv* mutation. Also, the BAC transgene containing whole *qkI* gene could suppress the tremor characteristic of the *qk* mutants. In addition, *qkI* expression was found to be reduced significantly in qkv homozygotes and hence was even less in the compound heterozygotes, qk^v/qkI^0. According to these results, we conclude that the *qkI* is the true responsible gene for the *qk* mutation, and that the reduced expression of *qkI* causes the neurological phenotypes of the *qk* mutants. Dosage-dependent effect of the *qkI* is highlighted by the analysis of the qk^v/qkI^0 compound mice; these mice exhibited severer phenotype than qk^v/qk^v including early onset seizures, gait abnormality and dwarfism. In many ways this phenotype resembles that of some of the human leukodystrophies, representing a novel mouse model for these dysmyelinating diseases. The severe phenotype found in the compound is probably due to premature arrest of myelination. The oligodendrocyte processes fail to interact properly with the axons to form compacted myelin, whereas the $qk^{v/v}$ oligodendrocytes can make loosely wrapped myelin sheaths. Expression analyses of several myelin marker genes at RNA and protein level suggest the involvement of *qkI* in post-transcriptional regulation or in alternative splicing processes of the myelin genes.

The *qkI* is also involved in embryonic development as the knock out homozygotes shows embryonic lethality; they die around e9.5 with an irregular shaped neural tube and disorganization in somites or heart. Therefore, the *qkI* gene plays important roles in multiple developmental processes such as neurulation or oligodendrocyte development.

E16. DOWNREGULATION OF SONIC HEDGEHOG (*Shh*) AND PATCHED (*Ptc*) EXPRESSION IN AN X-RAY INDUCED MOUSE MUTANT EXHIBITING LIMB AND FACIAL ABNORMALITIES.

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In vertebrates, limbs develop from paired buds at specific regions along the antero-posterior body axis. Limb bud development begins with the differentiation of the proximal cells whereas distal cells remain undifferentiated for a long time. Separated digits appear around day 12 p.c. as a consequence of controlled apoptosis. The growing limb is characterized by distinct spatial and temporal expression of growth and transcription factors. The centers are the posterior mesoderm zone of polarizing activity (ZPA), the limb ectoderm and the apical ectodermal ridge (AER). Members of the fibroblast growth factor family have been shown to induce limb outgrowth from the flank of the embryo. A major signaling gene in the induction of polarity and anteroposterior patterning is sonic hedgehog (*Shh*). The *Wnt-7a* gene has been identified as a factor necessary in ectodermal mediated dorsoventral signaling. A positive feedback loop between mesenchyme and ectoderm signaling coordinates patterning and outgrowth of the limb.

Linkage analyses of 606 backcross animals and 12 microsatellites suggest a 16 cM inversion of the proximal part of chromosome 5, which was confirmed by anaphase bridge analysis. These findings could disclose news genes involved in the anterior-posterior patterning of the limb.

In the offspring of x-irradiated mice we have recovered a mutant characterized by cataract, limb and cranio-facial malformations. They have a preaxial polydactyly on all 4 legs with the hindlimbs always being affected more evidently. The homozygous mutants, which die often during gestation, but sometimes survive to birth, exhibit additional phenotypic defects like soft tissue syndactylism on all four legs, additional pre- and postaxial digits of the forelimbs and a distinct hypognathy. Few homozygous offspring exhibit a cleft palate. Pathological examinations revealed partial heart ventricle and atrium defects.

Whole mount in situ experiments of embryos d11 p.c. revealed the reduced expression of *Shh* and its receptor *Ptc* in the limbs of heterozygous and no expression in homozygous mutants, whereas further expression patterns of *Shh* like notochord and of *Ptc* like somites are not involved. In heterozygous mutants the *HoxD13* expression is not affected but considerably reduced in the distal parts of the limb bud of homozygous mutants.

This mutant reveals an anterior-posterior limb patterning without expression of *Shh* and *Ptc* in the limb bud.

This work is supported by a grant of the DFG (WO 685/1).

E17. MORPHOLOGICAL AND DEVELOPMENTAL STUDY ON A NEW MUTANT KNOTTY-TAIL (*knt/knt*) MOUSE.

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The knotty tail mouse (gene symbol; *knt*) derived from the ICR strain has a short and kinked tail with a knot. Genetic data showed that knt was a simple autosomal recessive gene and its penetrance was complete. The caudal vertebrae were reduced in number and consisted of proximally normal ones and distally abnormal ones. Gross observation revealed that the anterior and posterior extremities of each deformed vertebral body were not parallel. Morphometrical observations of the caudal vertebrae indicated that the ratio of width to length of each vertebra shows a linear increase caudad in ICR strain, while it does not increase and is inconstant in the vertebrae below the 6th caudal vertebra. In time course examinations of the embryonal tail, somitogenesis was finished by day 12.0 pc, and the numbers of caudal somites more or less agreed with those of the caudal vertebrae in *knt/knt* mice. Somites below about the 6th caudal somite were wedge-formed with a dorsal apex in *knt/knt* embryos. The location of abnormal somites also well corresponded to that of deformed caudal vertebrae. From these results, it was suggested that the shortness of tail is primarily caused by the agenesis of distal caudal vertebrae following the agenesis of distal caudal somites, while the tail kinks are caused by the deformation of each caudal vertebra following disturbances of caudal somites.

E18. MULTIMERIC DNA BINDING COMPLEXES INVOLVING THE Meis1, Pbx AND Hox HOMEOPROTEIN FAMILIES IN MURINE MYELOID LEUKEMIA.

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The prototypical member of the *Meis1* homeodomain-containing gene family, *Meis1*, was originally identified as a common site of integration in virally induced myeloid leukemias in BXH-2 mice. The *Meis1* homeobox sequence is most similar to that of TALE superfamily of atypical homeodomain proteins and contains the three amino acid loop extension characteristic of this family. The most extensively studied TALE proteins so far are members of the *PBX/exd* family which recently have been shown to influence DNA binding affinity and site specificity through cooperative heterodimerization with a variety of homeodomain proteins, including a subset of the Hox proteins. The possibility that Meis1 may also function through interactions with other homeodomain proteins was suggested by the finding that almost 100% of BXH-2 tumors with *Meis1* integrations also had integrations at the *HoxA7* or *HoxA9* loci (Nakamura *et al.*, Nature Genet. 12:149-153, 1996).

We have begun to analyze the function of the Meis1 protein by examining its DNA binding properties and its potential interactions with other proteins. Binding site selection and electrophoretic mobility shift assay experiments show that the Meis1 proteins bind with high affinity to a specific palindromic recognition site and with much less affinity to a single half site. Mixing experiments with full-length Meis1 and Meis1 deletion constructs, as well as yeast two-hybrid analysis, suggest that these binding complexes contain homodimers that are capable of forming in the absence of DNA. Similar experiments, conducted with Meis1 in the presence of certain Hox proteins, have identified a specific binding site that consists of a hybrid between a Meis1 half site and a consensus Hox binding site. Although both Meis1 and Hox proteins could bind this site independently, gel shift experiments show that the Hox protein increases the affinity and stability of Meis1 binding (Shen *et al.*, Mol. Cell. Bio. 17:6448-58 (1997)). These results supported the idea that at least one mechanism of leukemogenesis in BXH-2 mice may involve coactivation of cooperating DNA binding proteins via viral integrations at *Meis1* and *HoxA9*.

Furthermore, recently published studies have indicated that members of the Meis1 family are capable of interacting with Pbx/exd, both in the cooperative binding of specific DNA recognition sequences, as well as part of a mechanism that facilitates Pbx/exd translocation from cytoplasm to the nucleus (Knoepfler *et al* ., PNAS 94:14553-58 (1997); Berthelsen *et al* ., EMBO J. 17:1423-33 (1998); Rieckhof *et al* ., Cell 91: 171-83 (1997)). Interestingly, western blot analysis of myeloid leukemia cell lines revealed that members of the Pbx family of proteins are expressed in tumors overexpressing *Meis1* and *HoxA7/A9*, suggesting that Meis/Pbx or Meis/Pbx/Hox interactions may also be important for transformation. We are currently investigating the relationship between expressed levels of *Meis1*, *Hox*, and *Pbx* gene family members and the nature of endogenous DNA binding complexes and preferred binding sites in cell lines derived from murine myeloid leukemias with viral integrations at *Meis1* and *HoxA7/A9* and results will be presented.

Research supported by NIH grant CA21124 (AMB).

E19. ANALYSIS OF THE qk^{k2} EMBRYO LETHAL ALLELE AND qk^{e5}, A NEW ENU INDUCED ALLELE OF QUAKING: THE DISCOVERY OF THE qk^{k2} MOLECULAR DEFECT AND A SEMIDOMINANT SEIZURE SUSCEPTIBILITY PHENOTYPE.

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The *quaking* gene, qkI, codes for a KH (RNA binding) domain protein with flanking QUA domains. The KH and QUA domains combined have been termed the "STAR" domain to define a growing family of "STAR" (Signal Transduction and Activation of RNA) proteins which contain the highly conserved central domain and features that suggest involvement in a signal transduction pathway (Reviewed in Vernet and Artzt, 1997).

Embryos homozygous for *qkk2*, an ethylnitrosourea (ENU) induced mutant allele of the *quaking* gene, die at approximately E9.5, exhibiting common defects of open neural tubes, abnormal somites and heart defects (Justice and Bode, 1988). *In situ* hybridization of embryos shows that homozygous qkk2 mutants have normal *brachyury* expression at E8.5 and E9.5, while *twist* is under expressed in the forebrain and first branchial arch. In addition to its recessive embryo lethality, *qkk2* exhibits a semidominant phenotype in adult heterozygous mice of reduced amounts of brain lipids and a high susceptibility to pentylenetetrazole (PTZ) induced seizures. Sequencing studies show that the defect in *qkk2* is a thymine to adenine transversion in the KH domain of qkI.

The *quaking* locus is complex and of the five known mutant *qk* alleles, no two are alike in the phenotypes that they produce as homozygotes, heterozygotes and compound heterozygotes. Consequently, each holds clues to specific *quaking* functions. We are therefore using ENU mutagenesis to produce new *qk* alleles to further our studies of *quaking* function. We are presenting our preliminary results including our new *qke5* allele, which we have begun to characterize.

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E20. DEVELOPMENTAL EXPRESSION OF JAGGED1 IN THE MAMMALIAN HEART: IMPLICATIONS FOR CARDIAC DISEASE IN ALAGILLE SYNDROME.

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Alagille syndrome (AGS) is an autosomal dominant disorder characterized by developmental abnormalities of the liver, heart, eye, skeleton and kidney. Congenital heart defects, the majority of which are right-sided, contribute significantly to mortality in AGS patients. Mutations in *Jagged1*, a conserved gene in the Notch intercellular signaling pathway, have been found to cause AGS. In order to understand the role of *Jagged1* in normal heart development and in the heart defects seen in AGS, we have studied the expression pattern of *Jagged1* in the developing mammalian heart.

Jagged1 expression was assayed by whole mount and section in situ hybridizations on mouse and human embryos. Whole mount in situ hybridizations of mouse embryos at 8.0 and 8.5 days post coitum (dpc), corresponding to 3 weeks of human gestation, reveal *Jagged1* expression in the first pharyngeal arch. *Jagged1* is expressed in the human heart at 8 weeks of gestation transmurally in the atria and in the endocardium of the ventricles. Expression is also detected in the epicardium, which is derived from liver primordium. In order to identify vascular structures in the mouse embryo at 10.5 dpc, in situ hybridization was performed with a probe for PECAM, an endothelial cell marker. Combined studies with probes for *Jagged1* and PECAM show overlapping patterns of expression in the branchial arch arteries and descending aorta, confirming *Jagged1* expression in these vascular structures. In situ hybridization of mouse embryos at 12.5 dpc shows *Jagged1* expression in the endothelial cells of the endocardial cushions which will undergo mesenchymal transformation to form valve tissue. Expression is particularly accentuated in the right-sided outflow tract, ductus arteriosus, pulmonary arteries and lung mesoderm.

We conclude that *Jagged1* is expressed in the developing mammalian heart in multiple vascular structures and in the developing valves. This pattern of expression provides evidence that *Jagged1* has a direct role in cardiac development and that the sites of expression correlate with the congenital heart defects seen in AGS.

E21. A COMMUNITY EFFECT IS RESPONSIBLE FOR A VARIEGATED COAT COLOR PHENOTYPE IN MICE.

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The « community effect » was defined by J. B. Gurdon as an interaction between cells of the same type, which is necessary for their entry in a differenciation pathway or for the completion of their differentiation. Community effects have been found experimentally in *Xenopus* (1-4), *Drosophila* (5), zebrafish (6) and ex vivo in the mouse (7). However, examples illustrating community effects in mammals remain rare.

patchwork (*pwk*), a spontaneous mutation identified by Karen Moore, causes coat colour mottling in mice. Animals homozygous for patchwork have a unique phenotype: they are variegated with white or fully pigmented hairs, but no partially pigmented (gray) hairs. If each hair follicle was derived from a single progenitor cell, one possible explanation for this phenotype could be that the mutation has an incomplete penetrance and/or expressivity. However, all components of a single hair are derived from more than one precursor. This holds true for the melanocytes and for the other hair follicle cells, as demonstrated using aggregation chimeras. To solve this apparent contradiction, we have investigated the etiology of the patchwork (*pwk*) mutation.

The target cell for the patchwork mutation is the melanoblast, as revealed by the coat colour pattern of aggregation chimeras between patchwork and albino embryos. These experiments also showed that patchwork does not act in a cell-autonomous manner. In patchwork fetuses, melanoblasts die by apoptosis from E18.5 in white hair follicles, as found following in situ cell death detection experiments. By contrast, the melanoblasts survive and differentiate normally in the pigmented hair follicles. The combination of patchwork and $Kit^{W\text{-}e_i}/+$, a mutation responsible for a reduced number of melanoblasts in the hair follicle, suggested that patchwork melanoblasts cannot survive and differentiate when they are in reduced number in the hair follicles (8). Thus, in patchwork fetuses, groups of few *pwk/pwk* melanoblasts die, but larger groups survive and differentiate into melanocytes.

patchwork seems to be involved in local interaction of the melanoblast with like neighbors in the hair follicle. The patchwork mutation affects the interaction so that the mutant melanoblast is subject to a community effect at E18.5. The molecular nature of the local signal is not yet known. In the melanoblast, the *pwk* gene is believed to control the production or the reception of an autocrine or paracrine signal whose concentration is crucial for melanoblast survival and/or differentiation. The identification of this product should help the understanding of the molecular and cellular bases for the community effects.

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E22. THE MOUSE AND HUMAN HOMOLOGS OF *Drosophila melanogaster neuralized* GENE ARE EXPRESSED DURING LIMB DEVELOPMENT.

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We have cloned the mouse and the human orthologs of the *D. melanogaster neuralized* gene. This gene is one of the so-called neurogenic genes, expressed in the early stages of D.m. neurogenesis determining the neural cell fate. Drosophila *neu* is expressed in the wing imaginal discs and sensory organ precursors. It encodes a protein containing a C_3HC_4 RING Zn finger, which has been characterized in a variety of regulatory proteins including transcription factors, locus-specific drosophila chromosomal proteins and oncoproteins. We used a human EST showing significant sequence similarity to the drosophila *neuralized* gene in order to screen a mouse and a human embryonal brain cDNA libraries. Sequencing of the isolated cDNAs revealed a predicted 574 aa polypeptide, containing a C_3HC_4 RING Zn finger motif at the carboxy-terminus, in both species. Both mouse and human proteins were found to share a 93.9% sequence identity and a 33% identity to the drosophila homolog, spread through out the sequence. Interestingly, the drosophila Neu polypeptide is longer (754 aa) than its mammalian counterparts. Protein alignments suggested three major gaps. However, the RING Zn finger domain has remained highly conserved as far as both sequence and topography is concerned. Sequence gaps may reflect additional characteristics of the drosophila molecule. Southern blot analysis suggested that the gene is unique in the mammalian genome, it is organized in five exons having a size of about 7 Kb in both species. The human gene maps to 10q24 and the mouse gene, possibly, to Chromosome 17 in a syntenic to chromosome 10 region. Northern blot analysis detected a major transcript of about 4 Kb. The expression profile of the gene was studied by in situ hybridisation in tissue sections of mouse embryo stages E9.5 through E16.5d. The gene showed a predominant uniform expression in the cartilagious limb skeletal precursors, vertebrae and ribs. Reverse transcription-PCR experiments using RNA samples from mouse embryonal limbs confirmed the above information. The same uniform expression pattern was detected in limb sections from, an approximately, six week human embryo. Our data suggest the isolation of a novel gene, the mammalian homolog of drosophila *neu*, exhibiting tissue-specific expression predominantly in early stages of limb development. The mechanisms of limb morphogenesis include a complex array of gene interactions. These processes are mediated by a variety of genes that are conserved between mammals and invertebrates. Identifying and characterizing more genes possibly involved in these interactions, will enable a better understanding of ongoing regulatory and pathogenic mechanisms.

E23. POSSIBLE INVOLVEMENT OF Mid1 IN THE PATCHY FUR (Paf) MUTANT.

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We have recently reported that X-linked Opitz-G/BBB syndrome (OS) is caused by mutations in the *MID1* gene (Quaderi et al., 1997, Nat. Gen. vol.17, pp285-289). OS is a multiple organ disorder primarily affecting midline structures. The cardinal manifestations of OS are hypertelorism and hypospadias. MID1 is a new member of the B-box family of proteins which are characterised by a tripartite motif consisting of a RING finger domain, one or two B-boxes and a coiled-coil domain. Human *MID1* maps to Xp22 and has no detectable Y homologue. In contrast, murine *Mid1* spans the pseudoautosomal boundary: the first three exons are X-linked whereas the remaining exons are present in the pseudoautosomal region (PAR) of both the X and Y chromosomes. (Palmer et al.,1997, PNAS 94(22), pp12030-12035; Quaderi et al., 1998, Hum. Mol. Gen. vol.7 no. 3, pp489-499). Patchy fur (*Paf*) is a semi-dominant X-linked mutation that produces an abnormal coat. Although OS and *Paf* have no common features, the observed delayed disjunction at meiotic metaphase I in *Paf* mutants may be attributed to improper pairing in the PAR due to chromosomal rearrangements involving the *Mid1* locus. Preliminary analyses suggest that *Paf* mice have a rearranged *Mid1* locus, and further studies are in progress. In addition, we are generating transgenic mice carrying a truncated *Mid1* gene in order to compare the phenotype to both OS and *Paf*.

E24. CHARACTERISATION OF X-LINKED DEVELOPMENTAL MUTANTS WITH CRANIOFACIAL ABNORMALITIES.

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In man, malformations of the face and skull occur with a frequency of >1 in 1000 live births. However, sparse pedigree data and genetic heterogeneity often complicate molecular genetic analysis and can cause major problems in clinical classification, and hence prognosis. It is possible to exploit single gene mutations in the mouse which are known to affect head morphology, to clone and characterise novel genes involved in craniofacial malformations. We are currently studying two X-linked semidominant phenotypes (tattered and broad-headed) and one X-linked recessive phenotype (wide-faced) to identify novel genes involved in head development.

Heterozygous females have patchy hyperkeratotic skin and have an abnormal head morphology. Tattered males die between mid and late gestation and examination of affected embryos revealed abnormalities in craniofacial, limb and intestinal development. We have localised the tattered gene (*Td*) to a <0.2cM interval between *Tcfe3* and *DXHXS7465e* in the proximal region of the mouse X chromosome. Broad-headed (*Bhd*) males die within a few hours of birth possibly due to asphyxiation resulting from skeletal abnormalities in the head and neck regions. We have positioned *Bhd* close to *Zfx* in the *DXMit45* to *DXMit16* region using two high resolution intraspecific backcrosses. Wide-faced (*wf*) males and homozygous females have a similar craniofacial appearance to heterozygous broad-headed females but are fully viable. In an intraspecific backcross, *wf* cosegregates with *DXMit50* which lies distal to *Agtr2* on the mouse X chromosome.

The cloning and characterisation of genes implicated in the craniofacial malformations present in these three X-linked mouse mutants will allow identification of potentially homologous human syndromes. The characterisation of these genes will also increase understanding of the process of mammalian skull development and of the molecular signals involved in bone formation.

E25. EXPRESSION OF *Msx1* IN DERMOMYOTOME AND ITS DERIVATIVES.

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Msx1 is one of the three mouse homeobox genes homologous to the Drosophila *msh* gene. Expression of *Msx1* takes place during development in regions of inductive interactions between ectoderm and mesoderm. In limb buds, we have shown that these interactions are required for *Msx1* gene activation.

To mutate the gene, we have inserted the *nlacZ* reporter gene in phase with *Msx1* coding sequences by homologous recombination in embryonic stem (ES) cells. Mutant mouse lines have been derived from four independent ES cell clones. Mutants die at birth showing no obvious phenotype in the limb. It is likely that at this site, the absence of *Msx1* is compensated by expression of the closely related *Msx2* gene.

Taking advantage of the high sensitivity of *nlacZ* detection, we have observed expression of *Msx1* in the lateral margin of the dermomyotome of some somites. Msx1-nlacz mouse somites were transplanted in the chick to follow nlacz transcription from the *Msx1* locus. We thus demonstrated that Msx1-positive cells migrate to the limb buds; co-expression of *Msx1* and *Pax3* in these cells confirmed that they are muscle precursors. Using the same strategy, we have shown that *Msx1* is expressed in dermis precursor cells derived from the dermatome. This makes Msx1 a new marker of early dermis and the first one to be specifically expressed in dorsal dermal precursors during their migration. *Msx1* is downregulated prior to the expression of genes specific for differentiated dermis or muscle, which fits with a role for Msx1 as a general repressor of differentiation.

E26. GENETIC ANALYSIS AND PHYSICAL MAPPING OF POLYDUCTYLOUS MOUSE MUTATION, HEMIMELIC EXTRA TOES (*Hx*).

T. Sagai, *H. Masuya, T. Shiroishi. Mammalian Genetics Laboratory, National Institute of Genetics, Yata 1111, Mishima Shizuoka-ken 411-8540, Japan.*

Many limb mutants are known in the mouse. Some of them have abnormal three axes (proximo-distal, antero-posterior and dorso-ventral) formation governed by particular signaling centers. One of the mutants, hemimelic extra toes (*Hx*) shows preaxial polyductyly on all four feet, associated with hemimelia, shortening of the radius, tibia and talus. *Hx* is located on the proximal region of chr 5 and is very closely linked to hammer-toe (*Hm*) mutation, which shows interdigital webbing regression probably due to an impairment of apoptosis. Because sonic hedgehog (*shh*) gene that mediates antero-posterior signaling has been also mapped to this region and ectopic expression of shh has been detected in the limb bud of *Hx*, it has been proposed that *shh* is a candidate gene for *Hx*.

We have mated the several laboratory and wild inbred strains with *Hx* heterozygotes and observed the phenotypes of the progeny. The progeny from different mating crosses exhibited characteristic phenotypes. In particular, hemimelia that characterized the original mutant phenotype reproducibly disappeared in the progeny from some crosses. The results suggested a possibility that genetic background of the strains modified the phenotype of *Hx* mutation. We are trying to fix these phenotypes by continuous backcross to the corresponding strains.

The linkage analysis of *Hx* mutation using 1570 backcross progeny from intersubspecific cross have mapped the candidate gene to a 0.6 cM segment. One of the recombinants could separate the coding region of *shh* from *Hx* gene, and excluded the *shh* coding as a candidate for *Hx* gene . We assume that there is at least one or two genes in this region in addition to *shh*, which may be responsible for the antero-posterior specification and the apoptotic cell death. In human, a complex bilateral limb deformity (Nicolei- Hamel polysynductyly) has been mapped to the syntenic region of chromosome 7q36. For the positional cloning of *Hx* and *Hm* genes, we have established a YAC contig covering the *Hx* region. The result showed that *Hx* and *shh* genes are colocalized within a 1.5 Mb single YAC clone and *Hx* gene is included in a 600 kb single YAC clone. The construction of BAC contig covering the 1.5 Mb DNA which includes *Hx* and *shh* genes is now under way.

E27. AN ALLELIC SERIES INDUCED BY ENU AT *axis* REVEALS MULTIPLE FUNCTIONS DURING DEVELOPMENT.

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The mouse is a powerful model organism to study mammalian gene function due to its many genetic tools and extensive comparative molecular linkage map with humans. To dissect the function of a gene, however, the phenotypes from an allelic series must be studied. Through chemical mutagenesis and elegant phenotypic screens, numerous mutant alleles (reflecting complete loss, partial loss, or gain of function) for any gene of interest can be derived.

For example, saturation mutagenesis with ethylnitrosourea (ENU) using the deletions at the *albino* locus on Chromosome 7 revealed many new functional units (Rinchik et al. 1990, 1995). In this phenotypic screen, a number of embryonic lethal mutations were isolated. Our focus is on one collection of mutants that define what we call the *axis* locus because it appears to affect the development of the body axis. The six isolated ENU-induced alleles of axis show a vast range in phenotypes. Two alleles arrest embryo development at a very early stage (primitive streak formation) and probably reflect the null phenotype. Two other alleles allow embryogenesis to proceed a little further but produce a disorganized body axis later in development. Another allele exhibits a variety of neural tube defects, including exencephaly. Interestingly, the final allele allows the mouse to survive to adulthood but shows subtle skeletal abnormalities. Taken together, the axis locus appears to influence the rostral-caudal body axis and neurulation. Complementation studies of these six alleles reveal complex genetic characteristics, including intragenic complementation between two of the lethal alleles and a parent of origin effect for the viable allele.

To access the gene mutated at this intriguing locus, we have generated a 580 kb BAC contig spanning the region. BAC sample sequencing has revealed a candidate gene that plays multiple roles during embryonic development and in the adult. The varied genetic features of *axis* -- which could only have been described by isolating an allelic series for this locus -- will provide important insights into the mechanism of gene function.

E.M. Rinchik, D.A. Carpenter & P.B. Selby. Proc. Natl. Acad. Sci USA 87, 896-900 (1990).
E.M. Rinchik, D.A. Carpenter & M.A. Handel. Genetics 92, 6394-6398 (1995).

E28. GENOME ANALYSIS OF THE MURINE *Pax1* DELETION MUTANT $Pax1^{Un\text{-}s}$ AND SEARCH FOR ADDITIONAL GENES INVOLVED IN THE $Pax1^{Un\text{-}s}$ MUTATION.

Bettina Wilm, *Rudi Balling, Ken-ichi Yamamura*, Kuniya Abe*, Kenji Imai. GSF-National Research Center for Environment and Health, Institute of Mammalian Genetics, 85764 Neuherberg, Germany; *Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan.*

Three natural *Pax1* alleles have been described of which two, $Pax1^{un\text{-}ex}$ and $Pax1^{Un\text{-}s}$, have been shown to represent deletion mutants with phenotypes of different severity. The skeletal phenotype of the $Pax1^{un\text{-}ex}$ allele very strongly resembles that of the $Pax1^{null}$ allele which was newly generated by gene targeting, indicating $Pax1^{un\text{-}ex}$ to be a natural null allele of the *Pax1* gene. In comparison, $Pax1^{Un\text{-}s}$ exhibits a skeletal phenotype which is much more severe, suggesting additional genes beside *Pax1* to be involved in its phenotype.

So far for both *Pax1* deletion mutants the sizes of the deletions were not known. We have therefore generated a BAC contig of the genomic region which surrounds the *Pax1* locus. By physical mapping, we could determine the size of the $Pax1^{Un\text{-}s}$ deletion to be 125 kb, with the *Pax1* gene being located approximately in the middle of the deletion. With the two overlapping BACs 132 and 604, the deletion can be completely covered.

The size of the deletion suggests that other genes might be located within the deletion. In order to identify genes which might be lost in the $Pax1^{Un\text{-}s}$ deletion mutant, we have started with cDNA selection experiments. We have used the two BACs which cover the deletion for hybridisation with RNA prepared from wild-type against homozygous $Pax1^{Un\text{-}s}$ embryos of 9.5 dpc. From a total of 120 clones we could recover, 44 represent putative cDNAs from the wild-type genomic DNA. Among these 44 clones, one could be identified as *Pax1* cDNA. Characterisation of the clones is in progress.

In order to determine whether the BACs 132 and 604 could rescue the $Pax1^{Un\text{-}s}$ and/or the $Pax1^{null}$ phenotype, we have generated transgenic mice using the individual BACs, respectively. We expect to gain information not only about genes which might fall into the deletion, but also about regulatory and promoter elements of the *Pax1* gene. As breedings are in process, no conclusions can be drawn to date.

F. Informatics

Abstracts - Posters

F1. INTEGRATION OF ESTS INTO THE MOUSE GENOME INFORMATICS DATABASES.

Judith Blake, *Richard Baldarelli, Ken Frazer, Jim Kadin, Mary Mangan, Rick Palazola, Joel Richardson, Steve Rockwood, Laura Trepanier, Martin Ringwald, Janan Eppig and the Mouse Genome Informatics Staff. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

F2. NEW TECHNOLOGY IN MOUSE GENOME INFORMATICS.

G.T. Colby, *R.M. Bardarelli, J.S. Beal, D.A. Begley, R.E. Blackburn, J.A. Blake, J.J. Bobish, D.W. Bradt, C.J. Bult, N.E. Butler, L.E. Corbani, G.L. Davis, M.T. Davisson, C.J. Donnelly, D.P. Doolittle, K.S. Frazer, J.C. Gilbert, L.H. Glass, P.L. Grant, J.A. Kadin, D.M. Krupke, M. Lennon-Pierce, L.J. Maltais, M.E. Mangan, M.E. May, M.G. McIntire, J.J. Merriam, J.E. Ormsby, R.P. Palazola, S. Ramachandran, D.J. Reed, J.E. Richardson, M. Ringwald, S.F. Rockwood, D.R. Shaw, L.E. Trepanier, P.G. Trepanier, H. Zhou, J.T. Eppig. The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

F3. MOUSE GENOME INFORMATICS: AN INTEGRATED RESOURCE FOR THE MOUSE COMMUNITY.

J.T. Eppig, *R.M. Baldarelli, J.S. Beal, D.A. Begley, R.E. Blackburn, J.A. Blake, J.J. Bobish, D.W. Bradt, C.J. Bult, N.E. Butler, G.T. Colby, L.E. Corbani, G.L. Davis, M.T. Davisson, C.J. Donnelly, D.P. Doolittle, K.S. Frazer, J.C. Gilbert, L.H. Glass, P.L. Grant, J.A. Kadin, D.M. Krupke, M. Lennon-Pierce, L.J. Maltais, M.E. Mangan, M.E. May, M.G. McIntire, J.J. Merriam, J.E. Ormsby, R.P. Palazola, S. Ramachandran, D.J. Reed, J.E. Richardson, S.F. Rockwood, D.R. Shaw, L.E. Trepanier, P.G. Trepanier, H. Zhou, M. Ringwald. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

F4. FINDING A MOUSE: TOWARDS AN INTERNATIONAL STRAIN RESOURCE.

Janan T. Eppig*, *Larry E. Mobraaten*, Muriel T. Davisson*, Carolyn S. Blake*, Simon Greenaway#, Rachael Selley#, Mark Strivens#. *The Jackson Laboratory, Bar Harbor, ME 04609, USA; #MRC Mammalian Genetics Unit, Harwell, Oxfordshire, OX11 ORD, UK.*

F5. IMPLEMENTING THE ANATOMICAL DICTIONARY IN THE GENE EXPRESSION DATABASE.

J.A. Kadin, *J.T. Eppig, D.A. Begley, G.L. Davis, K.S. Frazer, M.E. Mangan, R.P. Palazola, L.E. Trepanier, J.E. Richardson, M. Ringwald, and the Mouse Genome Informatics Staff. The Jackson Laboratory, Bar Harbor, ME 04609.*

F6. MOUSE DATA IN SWISS-PROT.

Michele Magrane, *Vivien Junker, Stephanie Kappus, Fiona Lang, Nicoletta Mitaritonna, Claire O'Donovan, Rolf Apweiler. EMBL Outstation – European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB1 1SD, UK.*

F7. MAP MANAGER XP, GENETICS MAPPING SOFTWARE FOR MICROSOFT WINDOWS AND MAC OS.

Kenneth F. Manly, *Robert H. Cudmore, Jr. Roswell Park Cancer Institute, Buffalo, NY, USA 14263-0001.*

F8. THE MOUSE GENOME DATABASE - MGD http://www.informatics.jax.org

J.J. Merriam *and the Mouse Genome Informatics Group Principal Investigator: Janan T. Eppig. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

F9. MOUSENET: CENTRAL DATA AND WORKFLOW MANAGEMENT FOR THE MUNICH ENU-MOUSE-MUTAGENESIS-SCREEN VIA THE INTERNET.

Walter Pargent*, Stephan Heffner*, *Dian Soewarto*, Andreas Teubner*, Birgit Rathkolb#, Eckhard Wolf#, Rudi Balling*, Martin Hrabe de Angelis*. *GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany; #Genecenter, Ludwig-Maximilians-University, 81375 Munich, Germany.*

F10. FROM EST SEQUENCES TO RADIATION HYBRID MAPS.

Jeremy Parsons, *Patricia Rodriguez-Tome. EMBL Outstation, the EBI, Wellcome Trust Genome Campus, Hinxton, Cambs CB10 1SD, UK.*

F11. MOUSE GENOME DATABASE – MAPPING RESOURCES.

Sridhar Ramachandran, *D.W. Bradt, J.J. Merriam, J.A. Blake, L.E. Corbani, P.L. Grant, J.J. Bobish, S.F. Rockwood, J.A. Kadin, J.E. Richardson, G.T. Colby, J.T. Eppig. The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

F12. DEFINITION OF HUMAN/MOUSE HOMOLOGY RELATIONSHIPS.

M.F. Seldin[1], *G.D. Schuler[2]. [1]Rowe Program in Genetics, Univ. of California, Davis, Davis CA; [2]National Center for Biotechnology Information, Nat. Inst. of Health, Bethesda, MD.*

F13. GENE EXPRESSION DATABASE FOR THE LABORATORY MOUSE.

David R. Shaw, *Joel E. Richardson, Dale A. Begley, Goeff L. Davis, Janan T. Eppig, Kenneth S. Frazer, James A. Kadin, Mary E. Mangan, Richard P. Palazola, Laura E. Trepanier, Martin Ringwald. The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

F14. INFORMATICS AT MRC HARWELL; CORBA AND JAVA A MODEL FOR DATA CAPTURE AND DISSEMINATION.

Hewitt M, Greenaway S, Mallon A-M, Selley R, Vizor L, Peters J, Brown SDM, **Strivens MA**. *MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell, Oxfordshire, UK.*

F15. RATACE: A DATABASE OF RAT cDNA GENE SEQUENCE.

David L. P. Vetrie, *Adam P. Butler, Sarah E. Hunt*, Carol E. Scott*, Liz A. Campbell, Clare L. East, Tom C. Freeman. Gene Expression and *Human Genetics Groups, The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, United Kingdom, CB10 1SA.*

F1. INTEGRATION OF ESTS INTO THE MOUSE GENOME INFORMATICS DATABASES.

Judith Blake, *Richard Baldarelli, Ken Frazer, Jim Kadin, Mary Mangan, Rick Palazola, Joel Richardson, Steve Rockwood, Laura Trepanier, Martin Ringwald, Janan Eppig and the Mouse Genome Informatics Staff. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

Information about ESTs, their putative identification, and the libraries they are derived from is eagerly sought by genome scientist. The Mouse Genome Database (MGD) incorporates mouse molecular segment data that include mouse EST information, descriptive details about probes, clones and PCR products, and links to sequence databases and other molecular resources. This information is carefully integrated with other information about mouse genes and genetic markers including details of mapping experiments and mammalian homology data. Regular updates of mouse EST data and curtain of associated information is accomplished through both automated and manually-curated processes. Links to the nucleotide sequence databases are provided as the gene information is described. Recently, links directly between an MGD marker and a Swiss_Prot protein record have been carefully annotated by MGD and Swiss_Prot editors. These annotations of molecular sequence data with other gene-based information enhance the usefulness of the Mouse Genome Informatics resources.

The strategy and mechanism of these data assocations within MGD and the Gene Expression Database (GXD) are the focus of this presentation.

MGD is supported by NIH grant HG00330. GXD is supported by NIH grant HD33745.

F2. NEW TECHNOLOGY IN MOUSE GENOME INFORMATICS.

G.T. Colby, *R.M. Bardarelli, J.S. Beal, D.A. Begley, R.E. Blackburn, J.A. Blake, J.J. Bobish, D.W. Bradt, C.J. Bult, N.E. Butler, L.E. Corbani, G.L. Davis, M.T. Davisson, C.J. Donnelly, D.P. Doolittle, K.S. Frazer, J.C. Gilbert, L.H. Glass, P.L. Grant, J.A. Kadin, D.M. Krupke, M. Lennon-Pierce, L.J. Maltais, M.E. Mangan, M.E. May, M.G. McIntire, J.J. Merriam, J.E. Ormsby, R.P. Palazola, S. Ramachandran, D.J. Reed, J.E. Richardson, M. Ringwald, S.F. Rockwood, D.R. Shaw, L.E. Trepanier, P.G. Trepanier, H. Zhou, J.T. Eppig. The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

There is a wide range of computing resources available within the mouse genetics community. While it is often tempting to quickly adopt the latest technology, we must consider the pros and cons before making a decision.

In the Mouse Genome Informatics group, we are often asked when we are going to commit to a new technology. Some examples include Java applets and applications, JavaScript, Windows NT, Sybase upgrades, WWW server upgrades, operating system upgrades, hardware and software upgrades.

Before adopting a new technology we must consider the following:

- Reliability
- Availability
- Compatibility
- Sustainability
- Usefulness
- Usability
- Cost
- Security

We will describe these issues in detail and examine several new technologies in order to understand why they are or are not used in the Mouse Genome Informatics project.

The Mouse Genome Database Project is support by NIH grant HG00330. The Gene Expression Database Project is supported by NIH grant HD33745.

F3. MOUSE GENOME INFORMATICS: AN INTEGRATED RESOURCE FOR THE MOUSE COMMUNITY.

J.T. Eppig, *R.M. Baldarelli, J.S. Beal, D.A. Begley, R.E. Blackburn, J.A. Blake, J.J. Bobish, D.W. Bradt, C.J. Bult, N.E. Butler, G.T. Colby, L.E. Corbani, G.L. Davis, M.T. Davisson, C.J. Donnelly, D.P. Doolittle, K.S. Frazer, J.C. Gilbert, L.H. Glass, P.L. Grant, J.A. Kadin, D.M. Krupke, M. Lennon-Pierce, L.J. Maltais, M.E. Mangan, M.E. May, M.G. McIntire, J.J. Merriam, J.E. Ormsby, R.P. Palazola, S. Ramachandran, D.J. Reed, J.E. Richardson, S.F. Rockwood, D.R. Shaw, L.E. Trepanier, P.G. Trepanier, H. Zhou, M. Ringwald. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

Two important resources for mouse genomic, gene expression, and phenotypic data and analysis exist, and continue to expand and develop at The Jackson Laboratory. These resources, the Mouse Genome Database (MGD) and the Gene Expression Database (GXD), have as their long term goal the facilitation of research through access to integrated genomic structural data, gene expression information, and phenotypic descriptions.

The Mouse Genome Informatics WWW site (http://www.informatics.jax.org) provides a method for transparent navigation of these integrated data resources for addressing complex questions of biological importance. In addition, this unified site will allow future expansion into other areas of mouse biological data, such as the mouse tumor information and enhanced disease model data.

MGD stores genomic structural and phenotypic data. These include gene definition, identification, and nomenclature; genetic, cytogenetic, physical, and comparative maps and data supporting those maps; clones/ESTs/probes/PCR characterizations and sequence links; allelic polymorphism data; mammalian homologies for 50+ species; descriptive phenotypic information for genes, mutations, and mouse strains; and reports of the mouse Chromosome Committees.

GXD will store and integrate data from the many types of expression assays, from large-scale high throughput methodologies, to gel-based assays (e.g. Northerns, Westerns), to detailed RNA or protein in situ analyses. Initial work is concentrated on expression in normal and mutant animals during embryonic development. Standardization of the anatomical nomenclature is being established to aid in describing expression domains.

The structural genomic and phenotypic data stored in MGD and the expression data stored in GXD will be described, emphasizing recently implemented changes and enhancements, and future plans.

MGD is supported by NIH grant HG00330. GXD is supported by NIH grant HD33745.

F4. FINDING A MOUSE: TOWARDS AN INTERNATIONAL STRAIN RESOURCE.

Janan T. Eppig*, *Larry E. Mobraaten**, *Muriel T. Davisson**, *Carolyn S. Blake**, *Simon Greenaway#*, *Rachael Selley#*, *Mark Strivens#*. **The Jackson Laboratory, Bar Harbor, ME 04609, USA; #MRC Mammalian Genetics Unit, Harwell, Oxfordshire, OX11 ORD, UK.*

Major resources for mutant mice and specialized strains and stocks exist at The Jackson Laboratory (US), the MRC Mammalian Genetics Unit (UK), Oak Ridge National Laboratory (US), and various European Mouse Mutant Archieve (EMMA) sites. In addition, new ENU and other chemically induced mutants, deletions, transgenics, and knock-outs/-ins are being generated in large systematic research programs. Queries about where various animals can be obtained are often fielded by each of our institutions, as well as worldwide queries being posted to the mouse electronic bulletin board, mgi-list, maintained by the Mouse Genome Database (MGD).

We are collaboratively developing an International Strain Resource Listing that will be available through our respective World Wide Web sites (The Jackson Laboratory at http://www.jax.org and the MRC, Harwell at http://www.mgu.har.mrc.ac.uk). This joint stock listing will include, for a particular genetic mutation or stock, the chromosome, gene symbol, specific allele, name of mutation, type of mutations, the holder (JAX or Harwell), and whether the stock is available 'frozen' or 'live' form. Hypertext links will take the user from a particluar stock listing to the site(s) where that stock is available for details on how to obtain the mouse of interest. Additional links will be provided from the gene symbol to the MGD data and description for that gene.

Once the initial system is thoroughly tested and working for The Jackson Laboratory and MRC Mammalian Genetics Unit sites, we will seek to expand the International Strain Resource Listing to include stocks available from other providers of unique mouse resources for scientific research. Our goal is to develop the International Strain Resource Listing as a fundamental look-up catalog of all available stocks and mutations, such that 'finding a mouse' can be done with a single World Wide Web search.

F5. IMPLEMENTING THE ANATOMICAL DICTIONARY IN THE GENE EXPRESSION DATABASE.

J.A. Kadin, *J.T. Eppig, D.A. Begley, G.L. Davis, K.S. Frazer, M.E. Mangan, R.P. Palazola, L.E. Trepanier, J.E. Richardson, M. Ringwald, and the Mouse Genome Informatics Staff. The Jackson Laboratory, Bar Harbor, ME 04609.*

The Gene Expression Database (GXD) is a database system for storing and integrating various types of gene expression data for the laboratory mouse. GXD is fully integrated with the Mouse Genome Database (MGD). Starting with the June 1998 release of MGI 2.0, GXD can store results from Northern and Western blot, RT-PCR, RNA in situ hybridization, and immunohistochemistry assays.

Expression results from these assays are annotated against a comprehensive anatomical dictionary of mouse development established by collaborative efforts with MRC Human Genetics Unit, Edinburgh and the University of Edinburgh[1]. This talk will present an overview of how the anatomical dictionary is handled in GXD including query semantics and positive and negative expression.

The anatomical dictionary is implemented as a collection of 28 hierarchies, 27 for the various Theiler stages of embryonic development and one for the adult mouse. For instance, the "ear" structure of stage 22 has subnodes "external ear," "inner ear," and "middle ear," and each of these is broken down subsequently. Expression results in the database have the form, "expression of gene x is detected/not detected in anatomical structure y via assay type z." Age is implicit in the structure reference since each structure is associated with a developmental stage. The hierarchy supports annotation of results at an accurate level of resolution based on the assay type and tissue samples used. For example, blot assays typically use coarse grained structures like "heart," while in situs may give more detailed results like "endocardial lining." Searches against the database can take advantage of the hierarchical structure. For example, queries for expression in "brain" will retrieve results annotated for "cerebral cortex." Additional information for each expression result includes the level of expression, defined by a controlled vocabulary, experimental details of the assay, and optional images of the gel, blot, or in situ hybridization.

GXD and MGD are accessible to the public at http://www.informatics.jax.org.

Supported by NIH Grants HG00330, HD33745, and HD08435.

[1]Edinburgh collaborators: J. Bard, R. Baldock, D. Davidson, M. Kaufman.

F6. MOUSE DATA IN SWISS-PROT.

Michele Magrane, *Vivien Junker, Stephanie Kappus, Fiona Lang, Nicoletta Mitaritonna, Claire O'Donovan, Rolf Apweiler. EMBL Outstation – European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB1 1SD, UK.*

SWISS-PROT is a curated, non-redundant protein sequence database which provides a high level of annotation and integration with other databases. It is supplemented by TrEMBL (Translation from EMBL Nucleotide Sequence Database) which was created to deal with the increasing number of sequences to be incorporated into SWISS-PROT from genome projects. TrEMBL is a computer-annotated database which contains translations of all coding sequences in the EMBL Nucleotide Sequence Database which are not yet in SWISS-PROT. The mouse is one of a number of model organisms which has been selected for priority annotation as it is the target of large-scale sequencing and mapping. This means that new sequences and updates to existing entries are added to the database as quickly as possible with a high level of annotation, cross-references to specialised databases are included in the entries and specific documents have been created to provide additional information for the user. SP-TrEMBL (Release 6) currently contains 3520 mouse entries which will be annotated by a curator before addition to the SWISS-PROT database which has 3220 mouse entries (Release 36). Each entry contains sequence data, citation information, taxonomic data, and, when available, information such as protein function, post-translational modifications, domains and sites, secondary and quaternary structure, similarities to other proteins, diseases associated with deficiencies in the protein, sequence conflicts and variants. The information in SWISS-PROT is added manually by a team of biologists and comes from a number of sources including scientific literature, direct submissions to SWISS-PROT, other databases such as ENZYME and PROSITE, transmembrane and signal prediction programs, and collaboration with external experts. TrEMBL entries are automatically annotated by a system which analyses sequences by comparison to the biochemically characterised well-annotated entries in SWISS-PROT to predict in a standardised way the functional properties of the TrEMBL entry. Cross-references to sequence-related databases and to specialised data collections such as the Mouse Genome Database (MGD), a database of genetic and biological mouse data, are also included, both in SWISS-PROT and TrEMBL.

F7. MAP MANAGER XP, GENETICS MAPPING SOFTWARE FOR MICROSOFT WINDOWS AND MAC OS.

Kenneth F. Manly, *Robert H. Cudmore, Jr. Roswell Park Cancer Institute, Buffalo, NY, USA 14263-0001.*

Map Manager XP is microcomputer program to assist mapping of Mendelian markers in backcrosses, intercrosses, and recombinant inbred lines. Versions are available for both Microsoft Windows and Mac OS operating systems. Map Manager XP is a completely rewritten version of Map Manager Classic (Map Manager v2.6.5). Like its predecessor, it provides a rich graphical interface for entry, editing, rearrangement, and display of genotyping data. It will analyze more types of crosses than Map Manager Classic, being able to analyze data that include dominant alleles and mixed dominant and codominant alleles. It will also analyze data that include mixtures of backcross and intercross segregation patterns.

Map Manager XP tests for linkage and calculates map distance by two-locus methods, displaying LOD scores for linkage and map distances with a choice of three mapping functions. It will search a dataset for loci which are linked according to user-defined criteria, and it will search for. It will partition a group of loci into linkage groups, and it will attempt to order a group of linked loci by systematically testing local permutations of the order.

Map Manager XP will be the foundation for software currently being developed for mapping quantitative trait loci. This software, called Map Manager QTX, which will also be available for both Microsoft Windows and Mac OS operating systems. The Map Manager family of software is described and distributed on the World Wide Web at http://mcbio.med.buffalo.edu/mapmgr.html. Development and distribution of Map Manager XP is supported by a grant from The Rockefeller Foundation.

F8. THE MOUSE GENOME DATABASE - MGD http://www.informatics.jax.org

J.J. Merriam *and the Mouse Genome Informatics Group Principal Investigator: Janan T. Eppig. The Jackson Laboratory, Bar Harbor, ME 04609 USA.*

The Mouse Genome Database (MGD) is a comprehensive database of mouse genetic and biological information. The Human Genome Initiative has emphasized the distinct importance of the mouse as a model system and has accentuated the necessity of a parallel organizational effort.

Since its public introduction on the World Wide Web (WWW) in 1994, MGD has provided researchers with a tool for quick and easy access to information on genetic loci with standardized **nomenclature, probe** and **marker** descriptions and resources, associated **phenotypic** information, **mapping data,** mammalian **homologies** covering more than 70 species, **inbred strain** and **polymorphism** information, and annual **Mouse Chromosome Committee Reports.** The latest release of the database supports an expanded **expression** data resource. A number of user-friendly query screens, which allow a range of selection options, are supported by an abundance of internal links that connect data in a logical and useful manner. Convenient external links to a number of other related databases expand the scope and versatility of MGD. Numerous mirror sites in Europe, Asia and Australia facilitate access to this information.

To date, more than 48,000 references with data on over 23,000 loci and 9,000 genes are represented in MGD. Data are updated continuously from the published literature, electronic submissions, and bulk data downloads. Integrity checks and automated reports assist the staff with quality control. The database continues to evolve to meet the changing needs of the community it serves.

Supported by NIH Grant HG00330.

User Support staff can be reached by Email: mgi-help@informatics.jax.org, phone: 207-288-6445, or FAX: 207-288-6132.

F9. MOUSENET: CENTRAL DATA AND WORKFLOW MANAGEMENT FOR THE MUNICH ENU-MOUSE-MUTAGENESIS-SCREEN VIA THE INTERNET.

Walter Pargent*, Stephan Heffner*, *Dian Soewarto*, Andreas Teubner*, Birgit Rathkolb#, Eckhard Wolf#, Rudi Balling*, Martin Hrabe de Angelis*. *GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany; #Genecenter, Ludwig-Maximilians-University, 81375 Munich, Germany.*

The Munich ENU-Mouse-Mutagenesis-Screen is a large scale animal breeding and screening project. It includes two animal breeding facilities and a number of screening groups in different locations.

Major challenges within a large scale project are workflow and data management.To address these problems we developed a database system which divides into three major compounds:

1. Being highly interactive the **Animal Management System** enables the animal caretakers to protocol any action in the mouse room to the database on-line. The database itself initiates actions by automatically generating electronic worklists. Electronic protocolling and supervision reduces the risk of errors and enables us to work more time and space efficient.

2. Within the **Sample Tracking System** sample taking lists are produced and/or modified. Additionally the storage (e.g. frozen sperm for biological archiving of strains) and shipping of samples is protocolled. Multiple samples can be taken from one individual animal. Thus the unique sample ids serve as a referential "glue" between animal ids and the results produced by the screens.

3. The **Result Documentation System** produces electronic worklists and hence investigation requests for the individual screens on the basis of sample taking lists. Such lists can be either directly loaded to laboratory analyzer machines or printed and used for manual work. Screeners can display the results produced by the other screening groups as well. Additional statistic functions are being developed.

Central data store is a relational database management system (RDBMS, Sybase Adaptive Server) running on a UNIX workstation. Database design and methods for data access are implemented with SQL (structured query language) as a standard data definition and data manipulation language.

Users connect to the central database via the internet on the basis of the Java Database Connectivity (JDBC) database driver. The user interface(s) are implemented as thin clients using JAVA technology. The software (including JDBC) is deployed just in time as Java bytecode. Hence client platform requirement is a web browser or applet viewer including a Java Virtual Machine (JVM) Version 1.1.3 or higher. Standard Java Foundation Classes (JFC) can/should be installed on the client to reduce software loading time. Java enabled internet browsers are common on modern workplace computers. Thus often no special installation will be needed at all and MouseNet is ready to run just by a mouse click to the internet.

This data system combines the functions of a management and workflow system for animal breeding facilities with a laboratory information and management system (LIMS).

F10. FROM EST SEQUENCES TO RADIATION HYBRID MAPS.

Jeremy Parsons, *Patricia Rodriguez-Tome. EMBL Outstation, the EBI, Wellcome Trust Genome Campus, Hinxton, Cambs CB10 1SD, UK.*

Whole genome radiation hybrid (RH) mapping provides a means to localise any Sequence Tagged Site(STS) (EST, genetic marker, or other genomic fragment) to a defined map position in the genome, via the polymerase chain reaction (PCR). In 1995, a database - the Radiation Hybrid database (RHdb, Lijnzaad et al., 1998) was set up at the EBI to maintain the results of such PCR experiments including, at that time, an international consortium working on the human genome. Now, with the availability of RH panels for the mouse genome (T31, McCarthy et al. 1997), and large-scale mouse EST sequencing it is possible to build a catalog of mapped mouse genes. To make the mapping efficient it is important to avoid effort wasted on mapping cognate sequences multiple times.

At the EBI we have implemented a complete workbench for mouse RH mapping:

1 - EST clustering (http://corba.ebi.ac.uk/EST) Redundancy within the EST databases is discovered by superclustering all known mouse mRNA sequences together. The cluster database and the progenitor alignment/overlap and sequence databases are all offered with CORBA interfaces and Java clients allowing access from anywhere in the World.

2 - RHalloc (http://wwww.ebi.ac.uk/RHdb/RHalloc): to assist in International cooperation, a database has been set up to help organize the EST allocations between groups. This database is feeds off the supercluster information.

3 - RHdb (http://www.ebi.ac.uk/RHdb): the radiation hybrid database offers a centralised site where all raw data information can be found. Statistics on the data are calculated daily and shown on the web pages. Java applets act as clients to access the information in a user-friendly way.

All these services are offered by public CORBA servers (http://corba.ebi.ac.uk) and the underlying software is portable and free.

F11. MOUSE GENOME DATABASE – MAPPING RESOURCES.

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The Mouse Genome Database (MGD) at The Jackson Laboratory is a community resource providing a comprehensive database for genetic and biological data on the mouse. MGD provides a wide range of mapping resources incorporating primary research data collected from scientific publications, community curated reports and electronically submitted data.

Mouse mapping data includes regular electronic data downloads of individual haplotypes from DNA mapping panel data sets. Graphical displays of genetic linkage maps can be generated selectively by using consensus map positions or data from specific mapping panel data sets. Cytogenetic mapping information derived from in situ hybridization experiments is used to generate map displays showing the chromosome divided into cytogenetic bands. Marker symbols are aligned to the right and linked to the detailed marker records. Integrated Whitehead/MIT linkage and physical maps are also available on line. On the physical map display, contigs are set against the backdrop of the MIT genetic map. MGD also provides a comparative mapping resource based on a curated homology dataset including over 5 mammalian species. Users have the ability to generate comparative map displays, Oxford Grid displays and composite listings of all mouse/human and mouse/rat homology data. For mammalian species with on-line genomic databases, corresponding hypertext links are provided.

Record for recombinant inbred and recombinant congenic strain distribution patterns (SDPs) are collected into composite reports which may be formatted as hypertext, tab-delimited text or MapManager files. Somatic cell hybrid data are presented in a table indicating the presence or absence of a particular marker or chromosome vs. the presence or absence of the marker being tested for linkage.

Users have the ability to search and retrieve specific data types, sort search results and print reports. MGD data are updated daily. MGD continues to evolve, expanding its data coverage, providing new data manipulation and display tools as well as promoting data integration with other scientific resources to meet the growing needs of the scientific community.

MGD is accessible via the WWW at http://www.informatics.jax.org

Supported by NIH grant HG00330.

F12. DEFINITION OF HUMAN/MOUSE HOMOLOGY RELATIONSHIPS.

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The further definition of human/mouse homology relationships requires increasing the resolution of the relative chromosomal positions of single copy sequences in both species. To provide a higher order map of these mouse/human homology relationships we have: 1) extensively reviewed physical and genetic mapping data in both species; and 2) initiated selective mapping of mouse ESTs corresponding to well mapped human ESTs. The integration and annotation of our homology database (including over 1900 single copy sequences mapped in both species defining 202 homology groups) with several thousand mapped human UniGenes and their putative mouse orthologues provides a scaffold for the current and future studies (http://www.ncbi.nlm.nih.gov/Homology/). A variety of strategies have been utilized in our initial efforts to identify mouse EST orthologues of mapped human ESTs for mapping studies and orthology annotations. Representative sampling suggests that nearly half of mapped human UniGene clusters can be used in computational algorithms to identify a highly sequence similar mouse EST. More than half of these pre-selected mouse ESTs meet stringent criteria for orthology (average similarity score of > 1000 and > 84% identity with their closest matching human EST over an average sequence length of > 300 bp) and are the best sequence match in comparisons performed in both directions. Unambiguous chromosomal positions of > 40 selected ESTs have been defined in a well-characterized gene rich mouse backcross mapping. These results have allowed additional resolution of homology breakpoints. Together, the data suggest that the understanding human/mouse homology relationships can be rapidly improved by this EST based strategy and provide additional infrastructure for cross species utilization of genomic and phenotypic information. The ability to construct and utilize virtual maps should facilitate functional genomics efforts.

F13. GENE EXPRESSION DATABASE FOR THE LABORATORY MOUSE.

David R. Shaw, *Joel E. Richardson, Dale A. Begley, Goeff L. Davis, Janan T. Eppig, Kenneth S. Frazer, James A. Kadin, Mary E. Mangan, Richard P. Palazola, Laura E. Trepanier, Martin Ringwald. The Jackson Laboratory, Bar Harbor, ME 04609, USA.*

The process of differential gene expression generates extraordinarily complex networks of gene and protein interactions. With its new focus on gene function and expression analysis, genome research is beginning to elucidate these networks to understand the molecular basis of human health and disease. The laboratory mouse will serve as a pivotal animal model in these studies. High throughput expression methods will make it possible to analyze in parallel the expression of thousands of genes in different tissues that can be derived from many different mouse strains and mutants. These experiments will provide initial, global insights into expression profiles and molecular pathways, and lead the way to more focused expression studies using Northern and Western blot, RT-PCR, RNA in situ hybridization, and immunohistochemistry assays. These different types of expression assays must be combined to determine what transcripts and proteins are produced by specific genes, and where, when, and to what extent these products are expressed at the cellular level.

The Gene Expression Database (GXD) is designed to store and integrate the many types of expression data for the laboratory mouse and to make the data freely and widely available in formats appropriate for thorough analysis. Expression patterns are described using a comprehensive anatomical dictionary established through collaborative efforts with the MRC Human Genetics Unit and the University in Edinburgh[1]. The dictionary models the anatomy hierarchically to allow for continuous refinement of the nomenclature system, to fit with the different resolution of analysis methods, and to facilitate user annotation of expression data. The standardized annotations of expression patterns are complemented with digitized images of original expression data that are indexed via the terms from the dictionary. GXD is being integrated with the Mouse Genome Database (MGD) to enable a global analysis of genotype, expression, and phenotype information. Extensive interconnections with sequence databases and with databases from other species will extend GXDís utility for analysis of gene expression information.

Expression data are and will be acquired from the literature by database editors, but primarily data will come via electronic submissions directly from research laboratories. These data and the Gene Expression Index, a searchable index into the expression literature for mouse development, and mouse cDNA data are accessible to the general public at http://www.informatics.jax.org/.

F14. INFORMATICS AT MRC HARWELL; CORBA AND JAVA A MODEL FOR DATA CAPTURE AND DISSEMINATION.

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As we approach the post-genomic era the increasing amounts of data being generated from a wide range of biological projects necessitate effective bioinformatics support for data capture, analysis and dissemination. The Informatics Group within the MRC Mammalian Genetics Unit plays a major role in these areas designing intranet analysis systems (e.g. for ENU Mutagenesis and EST mapping projects) as well as data dissemination to the community over the Internet (http://www.mgu.har.mrc.ac.uk).

We are engaged in a large scale, phenotype driven ENU Mutagenesis program (see abstract by Nolan *et al.*) to develop the range and depth of the mouse-mutant resource. To ensure the correct and efficient collection and analysis of data we have developed the 'Mutabase' distributed database system. Mutabase utilises a three-tier object technology, using Java and CORBA (Common Object Request Broker Architecture), to develop a multi user, distributed database system. The three tiers consist of:

- Primary tier - industrial class relational database system
- Middle tier - CORBA server
- Third tier - WWW-based client applets and stand-alone applications implemented in Java

Using this approach we have developed a range of client tools, from simple HTML form interfaces to complex interactive analysis tools written in Java. In addition, complex data acquisition and analysis tools are being developed for automatic locomotor activity cages and pre-pulse inhibition testing apparatus. This object-orientated approach facilitates the rapid production of new client tools by reusing various existing components and has the potential for many applications in the future.

The increased complexity and amount of data relating to areas of active research such as chromosomal anomalies, mouse imprinting regions and DNA microarrays, will require effective data management. We plan to develop tools, based on this object technology, to provide access to these and other datasets to researchers internally and to the wider community via the WWW. The CORBA and Java technologies represent one of the most effective ways of accessing diverse data sources and providing tools, of various levels of complexity, to exploit them.

F15. RATACE: A DATABASE OF RAT cDNA GENE SEQUENCE.

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Our experience of performing 'large-scale' gene expression studies on the mouse (see The Jackson Laboratory, Gene Expression Database, dataset ID J:46439), highlighted the need for a comprehensive, well-curated database of gene sequence as a central resource for carrying out such analyses. More recently the focus of the group has shifted to the rat, where the usefulness of the collection of rat cDNA, genomic and EST sequences in public databases (e.g. EMBL and GenBank) is limited. A particular problem is identifying which entries actually represent the same gene, as the emphasis is on the individual researcher to annotate their sequence entry, which has resulted in non-standardised nomenclature. In addition, even when it is possible to ascertain that more than one sequence entry is available for a particular gene, it is difficult to visualise the relationship between one entry and another, as well as recognise differences such as splice variants, sequencing errors and/or polymorphisms between them. To address these issues and to meet our needs to co-ordinate high-throughput gene expression studies in the rat, we are developing a cDNA sequence-based database named RATACE.

RATACE is based on the widely-used platform of acedb, a database originally developed for the *C. elegans* genome project. The cDNA sequence information in RATACE was obtained by retrieving EMBL entries for all the publicly-available rat genes, with the exclusion of ESTs. 5753 entries fitting this description were available at the time of the search (Jan. '98). Several rounds of sequence comparison under differing degrees of stringency has allowed sequences originating from the same gene to be identified and grouped. To date, of the original entries imported, 2182 fall into 863 groups containing two or more (up to 20) sequences representing one gene. The cDNA sequence currently in RATACE is therefore derived from as many as 4434 different genes, however this number is likely to change with further analysis. We have then attempted to rationalise the nomenclature for each gene, based on the description line of the original EMBL entries, with some of the standard rules of nomenclature applied. Where there is an identifiable orthologue in the mouse and/or human, we have tried to use the gene name listed by the MGD or GDB. In cases where more than one sequence entry represents the same gene, as identified by our sequence comparisons or by SWISSPROT, these entries have been given the same gene name. Individual sequences can be displayed graphically and manipulated using a variety of DNA sequence analysis tools. Alignments of groups of sequences originating from the same gene can be viewed in order to identify the coding sequence, splice variants, multiple polyadenylation sites, and small sequence differences. In addition, where there is a corresponding SWISSPROT entry for a particular gene sequence, it can be displayed graphically alongside homologous proteins from other species.

To our knowledge, RATACE is the first database which has attempted to systematically archive rat cDNA sequence. We are currently developing it as a tool for handling all the reagents and data required for, and generated by, large-scale expression/sequencing studies, such as RT-PCR, SAGE, microarrays and cellular analyses, in the rat. Through collaboration with others in the field, we hope that this database will serve as a public repository for rat sequence information. It is our intention that RATACE will also eventually incorporate EST cluster data, genome mapping information and will provide links to mammalian orthologues of rat genes.

G. Mutagenesis

Abstracts - Posters

G1. ARCHIVING MOUSE MUTANTS BY SPERM FREEZING.

Susan Bartmann, *Ulrike Huffstadt, Rudi Balling, Martin Hrabé de Angelis. GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany.*

G2. GENOME FUNCTION IN THE MOUSE BY COMBINING PHENOTYPE-BASED MUTAGENESIS SCREENS.

Maja Bucan*, *Andreas Lengeling*, Gillian Leach*, Lisa Tarantino*, John Schimenti^. *Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; ^The Jackson Laboratory, Bar Harbor, Maine 04609.*

G3. THE DYSMORPHOLOGY SCREEN WITHIN THE MUNICH-ENU-MUTAGENESIS PROJECT - FIRST 93 MUTANT LINES.

Helmut Fuchs*, *Dian Soewarto*, Andreas Teubner*, Birgit Rathkolb#, Walter Pargent*, Stephan Heffner*, Eckhard Wolf#, Rudi Balling*, Martin Hrabé de Angelis*. *GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany. #LMU, Institut für Molekulare Tierzucht und Haustiergenetik, 81375 München, Germany.*

G4. CRYOPRESERVATION OF MOUSE SPERMATOZOA.

Peter Glenister, *Claire Thornton. Medical Research Council, Mammalian Genetics Unit, Harwell, OX110RD, UK.*

G5. THE HARWELL FROZEN EMBRYO BANK.

Peter Glenister, *Claire Thornton. Medical Research Council, Mammalian Genetics Unit, Harwell, OX110RD, UK.*

G6. AN EMBRYONIC STEM CELL DELETION BANK FOR PRODUCING DELETION COMPLEXES IN MICE.

Neal C. Goodwin, *J. Catherine Kneece, John C. Schimenti. The Jackson Laboratory, Bar Harbor, Maine.*

G7. PUTATIVE ASSIGNMENT OF EST's TO THE GENETIC MAP BY USE OF THE SSLP DATABASE.

Bruce J. Herron, *George H. Silva, Lorraine Flaherty. Molecular Genetics Program and Laboratory of Developmental Genetics, Wadsworth Center, Albany, NY 12201-2002, and the Department of Biomedical Sciences, State University of New York at Albany.*

G8. DEVELOPMENT OF LARGE-SCALE CHROMOSOMAL DELETION STRATEGIES FOR FUNCTIONAL GENOMIC ANALYSIS.

Karen A. Johnstone, *Andrew J.H. Smith. Centre for Genome Research, University of Edinburgh, Edinburgh, EH9 3JQ.*

G9. A DOMINANT SCREEN FOR NOVEL REST: ACTIVITY MUTANTS IN THE MOUSE.

David Kapfhamer, *Gillian Leach, Alireza Alavizadeh, Patrick Nolan, Maja Bucan. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.*

G10. A COMPREHENSIVE ENU MUTAGENESIS PROGRAMME FOR THE MOUSE GENOME.

Pat Nolan[1], *Jo Peters*[1], *Lucie Vizor*[1], *Claire Thornton*[1], *Pete Glenister*[1], *Simon Greenaway*[1], *Mazda Hewitt*[1], *Rachael Selley*[1], *Mark Strivens*[1], *Jo Martin*[2], *Elizabeth Fisher*[3], *Derek Rogers*[4], *Jim Hagan*[4], *Sohaila Rastan*[4], *Mick Browne*[4], *Jackie Hunter*[4], *Steve Brown*[1]. [1]*MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell, UK* [2]*Department of Morbid Anatomy, Queen Mary and Westfield College, London, UK.* [3]*Neurogenetics Unit, Imperial College, London, UK.* [4]*SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, UK.*

G11. PRODUCTION OF MOUSE MODELS WITH CLINICALLY RELEVANT PHENOTYPES BY ENU MUTAGENESIS.

B. Rathkolb[a], *E. Fuchs*[b], *I. Renner-Müller*[a], *D. Soewarto*[c], *A. Teubner*[c], *W. Pargent*[c], *S. Heffner*[c], *H. Kolb*[b], *R. Balling*[c], *M. Hrabé de Angelis*[c], *E. Wolf*[a]. [a]*Lehrstuhl für molekulare Tierzucht und Haustiergenetik, LMU München.* [b]*Institut für klinische Chemie, Städtisches Krankenhaus Harlaching, München.* [c]*Institut für Säugetiergenetik, GSF-Forschungszentrum, München.*

G12. NEW MOUSE MODELS FOR GENETIC DEAFNESS: A SYSTEMATIC APPROACH TO UNDERSTANDING THE MOLECULAR AND BIOLOGICAL BASIS OF DEAFNESS.

Karen P. Steel[1], *Amy Kiernan*[1], *Rudi Balling*[2], *Martin Hrabé de Angelis*[2], *Jean-Louis Guenet*[3], *Karen B Avraham*[4], *Jo Peters*[5], *Steve D M Brown*[5]. *1. MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, UK; 2. Institut für Säugetiergenetik, GSF-Forschungszentrum, Postfach 1129, Neuherberg, Oberschleißheim, D-85758, Germany; 3. Unité de Génétique des Mammifères, Institut Pasteur, 25 rue du Docteur Roux, Cedex 15, Paris, F-75015, France; 4. Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel; 5. MRC Mammalian Genetics Unit, Harwell, Didcot, Oxfordshire OX11 0RD, UK.August 24,1998*

G13. ANALYSIS OF FATTY ACIDS IN MICE USING GC/MS.

Lisa S. Webb[1], *Gary A. Sega*[1,2], *Dabney K. Johnson*[1,3]. [1]*The University of Tennessee Graduate School of Biomedical Sciences;* [2]*Chemical and Analytical Sciences, Division,* [3]*Life Sciences Division; Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077.*

G14. A FUNCTIONAL GENETIC ANALYSIS OF THE *brown* DELETION COMPLEX.

John S. Weber[1], *Eleanor H. Simpson*[2], *Ian J. Jackson*[2], *Karen Goss*[3], *Dabney K. Johnson*[3], *Andrew Haynes*[4], *Paul Denny*[4], *Steven D.M. Brown*[4], *Monica J. Justice*[1]. [1]*Dept. of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030,* [2]*MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU,* [3]*Life Sciencies Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8077,* [4]*MRC Mouse Genome Centre, Harwell, Didcot Oxfordshire, OX11 ORD.*

G1. ARCHIVING MOUSE MUTANTS BY SPERM FREEZING.

Susan Bartmann, *Ulrike Huffstadt, Rudi Balling, Martin Hrabé de Angelis. GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany.*

Due to the similarity in genomes, developmental and biochemical pathways and physiology of man and mouse, the mouse has become a major model system to study genetics, and pathogenetics of human diseases. In this context a mouse mutagenesis screen has been started at the GSF research center in Munich-Neuherberg to produce new mouse mutants by chemical mutagenesis.

With the increasing number of new mutants it has become necessary to preserve and archive the genetically valuable mutant strains.

Today cryopreservation of germplasm is well recognized for providing an efficient management of genetic resources. Especially sperm cryopreservation has become indispensable because sperm from a single male could potentially give rise as many as 20 times more offspring than embryos from a single female, and also in order to preserve strains in which female reproductive problems are characteristic.

Meanwhile a sperm-freezing-unit has been build up at the GSF research center. Associated with the freezing and thawing of mouse spermatozoa the method of in vitro fertilization of mouse oocytes is established. A study was conducted to show the reliability of the freezing method. The spermatozoa of 20 males (10 wildtype and 10 mutant animals) were frozen in 8-10 aliquots per male (15 µl each). After a certain time period (7-90 days) individual samples were thawed and an in vitro fertilization was made. Ooocytes which developed to two-cell embryos were transferred into pseudopregnant females. Only a small amount of the frozen sperm samples of one male was necessary to get a minimum number of 20 offspring. The results show, that archiving mouse mutants is reliably possible using sperm freezing technology.

Mouse mutants found in the Munich-ENU-mutagenesis screen will be archived using this technology and provided to the scientific community.

In future the sperm-freezing-unit will be supplemented by ovary cryopreservation.

G2. GENOME FUNCTION IN THE MOUSE BY COMBINING PHENOTYPE-BASED MUTAGENESIS SCREENS.

Maja Bucan*, *Andreas Lengeling*, Gillian Leach*, Lisa Tarantino*, John Schimenti^. *Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; ^The Jackson Laboratory, Bar Harbor, Maine 04609.*

Significant progress has been made in sequencing the genomes of several model organisms, and efforts are now underway to complete the sequencing of the human genome. In parallel with this effort, new approaches are being developed for the elucidation of the functional content of the human genome. Classical genetic approaches to gene function, such as phenotype-based mutagenesis screens are aimed at the establishment of a large collection of single gene mutations affecting a wide range of phenotypic traits in the mouse, and will play an important role in this phase of the genome project.

To maximize the efficiency of a mutagenesis screen, we are combining two screens: a) a genome-wide screen for dominant behavioral mutations and b) region-specific saturation screen for mutations within a proximal portion of mouse chromosome 5 (syntenic to 4p16 - 4q12 and 7q36 regions in the human genome). Our screen for behavioral mutations involves the following set of assays: "zero" maze, rotarod, analysis of the acoustic startle response (ASR) and inhibition of the ASR, wheel running activity and EEG/EMG scoring of sleep patterns. The region-specific screen is performed by combining high efficiency ENU mutagenesis with deletion complexes generated using embryonic stem (ES) cells (You et al., 1997) and an existing deletion (*W19H*). Deletion complexes are being generated around well defined loci (*Dpp6; Hdh, Qdpr, Gabrb1*) and a set of 5-7 large deletions spanning a 30 cM segment (*Rw* inversion) will be initially used in crosses with ENU mutagenized males. We will present an overview of our screens and a list of 4 characterized mutations, as well as those in the initial stages of the characterization.

The ability to create and analyze deletion complexes rapidly, as well as to map novel chemically induced mutations within these complexes, will facilitate systematic functional analysis of the mouse genome and corresponding gene sequences in humans. Furthermore, since the extent of the mouse genome sequencing effort is still uncertain, we underscore a necessity to direct any limited sequencing efforts to those particular chromosomal regions that are targets for extensive mutagenesis screens.

G3. THE DYSMORPHOLOGY SCREEN WITHIN THE MUNICH-ENU-MUTAGENESIS PROJECT - FIRST 93 MUTANT LINES.

Helmut Fuchs*, *Dian Soewarto**, *Andreas Teubner**, *Birgit Rathkolb#*, *Walter Pargent**, *Stephan Heffner**, *Eckhard Wolf#*, *Rudi Balling**, *Martin Hrabé de Angelis**. **GSF Research Center, Institute for Mammalian Genetics, 85764 Neuherberg, Germany. #LMU, Institut für Molekulare Tierzucht und Haustiergenetik, 81375 München, Germany.*

Phenotypic and genotypic analysis of mouse mutants has proven as an extremely successful approach towards the molecular understanding of morphological abnormalities and their genetic basis. To identify a large number of genes, an ENU-mouse-mutagenesis screen has been set up. The screen is one of the projects within the German Human Genome Project. A dysmorphology screen has been established to generate and characterize new mouse mutant lines with morphological abnormalities in different organ systems. The screen evaluates defects of the central nervous system (CNS), sense organs, limbs, axial skeleton and pigmentation. Presently focusing on dominant phenotypes a total of 16000 F1 offspring from mutagenized males have been screened (C3HeB/FeJ).

Currently 39 parameters are checked on every animal and the list of parameters in the dysmorphology screen is still growing. 363 variants have been isolated and tested in confirmation crosses to genetically confirm the variation. 116 confirmation crosses are still running, 19 variants died during breeding and 54 variants never produced offspring. 81 variations could not be genetically confirmed. So far we were able to generate 93 confirmed mutant lines. The 93 mutant lines include phenotypes like: kinky tails, double toes or other limb abnormalities; deafness, circling behaviour and head tossing. Some mutants are hairless, have abnormal skin or defects in pigmentation. Other mutant lines show cataracts, defects in tooth development and coat colour abnormalities. The initial characterisation of certain mutant lines is underway. Breeding the mutated alleles to homozygousity has been started. Subsequent mapping of the mutant gene loci will allow us to identify genes that are possibly involved in the establishment of human congenital diseases. A www page is under development to present the mutant lines to the scientific community.

G4. CRYOPRESERVATION OF MOUSE SPERMATOZOA.

Peter Glenister, *Claire Thornton. Medical Research Council, Mammalian Genetics Unit, Harwell, OX110RD, UK.*

Considerable effort has been expended at Harwell, since March 1997, to develop techniques of mouse sperm cryopreservation. This research was prompted by the initiation of a large new ENU mutagenesis programme for which it is proposed that DNA and sperm from all the male offspring of mutagenised males will be archived for future screening. This will involve freezing sperm from several thousand mice per year. It is essential that any technique adopted must be rapid, simple and reproducible. We are freezing sperm in a mixture of 18% raffinose and 3% skim milk according to the method of Okoyuma et al (1990). J. Fertil. Implant (Tokyo), 7, 116., Nakagata and Takeshima, (1992). Theriogenology, 37, 1283., and Sztein et al (1997). Cryobiology, 35, 46.

The main objective for sperm freezing was to archive (C3H/HeH xBALB/c) F1 progeny from the mutagenesis programme, therefore we have concentrated chiefly on freezing sperm of this genotype.

The vas deferens and cauda epididymes of individual males are minced in 1ml 18% raffinose, 3% skim milk and the sperm is allowed to disperse for 10min at 37oC. 100 - 200: l aliquots of the resulting sperm suspension are loaded into 1.5ml cryotubes and placed in liquid nitrogen vapour for 10 minutes. The samples are then plunged and stored in liquid nitrogen. The sperm is thawed rapidly by placing the cryotubes directly into a water bath at 37°C. The cryoprotectant is removed by centrifugation, the sperm re-suspended in IVF culture medium (modified MEM) and allowed to disperse for 10 min at 37°C. It is then used for in vitro fertilisation of freshly ovulated oocytes.

Several hundred oocytes (C3H/HeH x 101/H) F1 can be used per sperm sample for each IVF. To date, results are very encouraging. For example, using 1 x 100 l frozen sperm sample from one (C3H/HeH x BALB/c) F1 male, 144 mice were born. As 10 x 100 l aliquots were originally frozen, it is possible that we could recover ~1500 live-born offspring from the frozen sperm of this particular male. This level of success is reproducible in that similar results were obtained from the frozen sperm of 8 different males of the same genotype. Research is ongoing to ascertain whether the same success is achievable with different strains.

G5. THE HARWELL FROZEN EMBRYO BANK.

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The frozen embryo bank at the Mammalian Genetics Unit (MGU) Harwell, is the largest in Europe and one of the largest in the world. It contains many mouse mutants that are models of human genetic disease. The bank was founded in the mid 1970's after extensive studies into the feasibility of embryo freezing as a reliable and economical means of storing mouse mutants for long periods of time. It provides ready, economical and secure access to the increasing array of mutants arising from mutagenesis and transgenic programmes both within the MGU and from other mouse genetic centres in the UK and world-wide. Recently, the bank has expanded to offer researchers from external institutes the opportunity to have stocks frozen and banked at Harwell. The bank now acts as a central resource for the deposition and distribution of mouse mutants and stocks that are pivotal to much of today's genetic research. This service will become increasingly important as Harwell's role as a node for the new European Mouse Mutant Archive (EMMA) facility, Monterotondo, Italy develops. At present, the MGU frozen embryo bank contains more than 250,000 embryos comprising about 800 stocks. Cryopreservation of mouse sperm has recently been trialled at Harwell with considerable success and this new capability will become a valuable adjunct to the existing embryo banking facility. The MGU Harwell web site can be accessed at: http://www.mgu.har.mrc.ac.uk

G6. AN EMBRYONIC STEM CELL DELETION BANK FOR PRODUCING DELETION COMPLEXES IN MICE.

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Deletion mutation analysis is a valuable tool that can be exploited to assign functional importance to large genomic regions. The inability to create large deletion complexes in the mouse genome has been a limitation in the implementation of this tool. Recent technology has been developed that overcomes this limitation by using γ-irradiation of mouse embryonic stem (ES) cells to produce deletions, and the subsequent transmission of these ES cells into the germ line to produce deletion complexes in mice. This technology requires targeting specific loci with a reporter cassette that provides both a positive selectable marker (*neo*) for the targeting event, and a negative selectable marker (*tk*) for the deletion event. A limitation in this procedure is that it requires the construction of specific targeting plasmids, and to perform deletion mutation analysis on a genome-wide scale, researchers must independently target hundreds of loci. The research presented here describes the production of an ES cell deletion bank (DelBank) that is comprised of individual ES F1 hybrid cell lines that differ from one another by the position of a reporter cassette within the genome. The DelBank was produced by transfecting ES cells with a reporter cassette, screening individual cell clones that contained single-copy integrations, and mapping the integration loci to approximately one centiMorgan resolution. Together these cell lines can be used to produce overlapping deletion complexes on a genome-wide basis.

G7. PUTATIVE ASSIGNMENT OF EST's TO THE GENETIC MAP BY USE OF THE SSLP DATABASE.

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Simple sequence length polymorphisms (SSLP's) have become standard mapping tools in mouse genomics. More than 6000 SSLP's have been mapped and their characteristics, sequences and map positions are available. We have used the Basic Local Alignment Search Tool (BLAST) and the flanking sequences of these SSLP's to identify the genetic positions of expressed sequence tags (EST) and of characterized genes that are now being deposited in available data banks. The identity of these EST's will aid in the clarification and usefulness of the mouse genome map.

G8. DEVELOPMENT OF LARGE-SCALE CHROMOSOMAL DELETION STRATEGIES FOR FUNCTIONAL GENOMIC ANALYSIS.

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We are developing genome engineering strategies in ES cells based on the application of Cre/loxP recombination in order to facilitate the functional analysis of the mouse genome. In one approach, we are using an ES cell line with ligand inducible Cre recombinase activity to generate deletions of large genomic regions flanked by loxP sites. The loxP sites are introduced by sequential targeting with vectors that have been modified to allow the genomic interval to be recovered. This is designed to simplify subsequent mapping and mutational analysis of the region.

G9. A DOMINANT SCREEN FOR NOVEL REST: ACTIVITY MUTANTS IN THE MOUSE.

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In a search for genes that regulate rest:activity and circadian behavior in mice, our lab is conducting a (semi) dominant, genome-wide screen in which progeny of mice treated with N-ethyl-N-nitrosourea (ENU) are screened for abnormal activity parameters (wheel-running seriod in constant dark, tentrainment to the light:dark schedule, and general organization of activity and rest phases). To date, several mutants have been identified in the (semi) dominant screen: *Wheels* (*Whl*), *Earlybird* (*Ebd*), and *Esterline* (*Est*). *Wheels* heterozygote mice are characterized by hyperactivity and circling behavior that is associated with neurodevelopmental anomalies and is embryonic lethal in homozygotes. *Wheels* maps to the proximal portion of chromosome 4. *Earlybird* and *Esterline* exhibit an abnormally shortened period of activity (greater than 3 standard deviations from the mean for wild-type). *Earlybird* heterozygotes display a period between 22.5 and 23.1 hours (compared to 23.7 hours for wild-type C57BL/6J mice), while ther period of *Ebd/Ebd* homozygotes ranges from 22.0 to 22.5 hours (on a pure C57BL/6J background). On a C3H/He/C57BL/6J background, the period of *Ebd/Ebd* mice is not significantly shorter than heterozygotes. In addition to the short circadian period, some homozygotes exhibit an advanced phase of activity onset relative to the light-dark transition and/or a high level of activity during subjective day. *Earlybird* maps to the proximal portion of mouse chromosome 8 in a region syntenic to human chromosome 4. The most attractive candidate gene in this critical region is the melatonin receptor (*Mtnr1a*), of which the potential relevance to *Earlybird* is under investigation.

G10. A COMPREHENSIVE ENU MUTAGENESIS PROGRAMME FOR THE MOUSE GENOME.

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Systematic approaches to mouse mutagenesis will be vital for future studies of gene function. Mouse mutants are available for only a small percentage of the total number of mammalian genes - there is a phenotype gap, (Brown and Peters, TIGS, 12: 433) and we need to increase both the breadth and depth of the mouse mutation resource. ENU mutagenesis represents an efficient phenotype-driven approach to deriving new mutations for the mouse mutant catalogue (Martin and Fisher, TIGS, 13: 254). We have begun a major ENU mutagenesis programme that incorporates a new systematic and semi-quantitative screening protocol - SHIRPA (Rogers et al., Mammalian Genome, (1997) 8(10): 711). SHIRPA is a hierarchical screening protocol employing a rapid and efficient primary screen for deficits in muscle and lower motor neurone function, spinocerebellar function, sensory function, neuropsychiatric function and autonomic function. Subsequently, secondary and tertiary screens of increasing complexity can be employed on animals demonstrating deficits in the primary screen. The mutagenesis programme will also provide hotel facilities for other members of the community to introduce efficient and integrated screens.

Spanning the next three years, two large-scale mutagenesis screens for viable mutations are in progress:

- a genome-wide screen for dominant mutations. Mutagenised BALB/c males are being mated to C3H and 40,000 F1 progeny characterised using the SHIRPA protocol.
- targeted screen for recessive viable mutations at the del36H deletion on mouse chromosome 13. This deletion region is homologous to human chromosome 6p22-23 and takes advantage of the sequencing and gene characterisation efforts currently focused on this chromosome.

We are developing an interactive, client/server database system using a WWW client (Sybase RDBMS, WebSQL, Java & CORBA components) for analysing screening data, tracking of tissue samples, embryo banking, sperm freezing operations as well as reports on mutant production and the performance of the SHIRPA protocol (see abstract by Hewitt et al.). For further information on the project and details of data derived from the screening see: http://www.har.mrc.ac.uk/mutabase/

To date, over 7,000 mice have been generated in the genome-wide screen of dominant mutations and a wide range of novel phenotypes recovered.

Around 1 in every 100 progeny are carrying heritable dominant mutations and examples of novel mutations and phenotypes that have been identified will be reported.

G11. PRODUCTION OF MOUSE MODELS WITH CLINICALLY RELEVANT PHENOTYPES BY ENU MUTAGENESIS.

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Mouse mutants are important tools to unravel molecular mechanisms of human disease. In the framework of a large-scale ENU-mouse-mutagenesis-screen funded by the German Human Genome Project, we perform a first line clinical and clinical-chemical investigation of mice derived from mutagenized founder animals. The screen includes clinical examination and basic hematological and clinical-chemical parameters which are suitable to detect altered functions of various organ systems and metabolic pathways. Blood samples are taken from fasted 3-month-old mice. The individual parameters are listed in Table 1. In addition, differential blood counts are performed.

Table 1: List of parameters measured in the clinical-chemical screen

Basic hematology	No. of red blood cells, white blood cells, platelets, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), hemoglobin concentration (MCHC), volume (MCV)
Plasma enzyme activities	Alkaline phosphatase, alanine-aminotransferase, aspartate-amino-transferase, α-amylase, creatine kinase
Plasma metabolites	Total protein, cholesterol, triglycerides, glucose, uric acid, urea, creatinine
Plasma electrolytes	Potassium, sodium, chloride, calcium, anorganic phosphate

So far, 1610 C3HeB/FeJ mice derived from ENU-mutagenized males were screened. A total of 161 of these mice showed altered levels in one or more parameters at the first measurement. Until now a second sample of 108 of these mice was tested for the altered parameters and in 32 cases the variation of at least one parameter was confirmed. Ten of these variants show alterations of hematological parameters, mostly of the MCV, eleven exhibit altered plasma enzyme activities and eleven are characterized by changes in plasma metabolite levels. No variants of plasma electrolytes have been found so far. In one case, elevated levels of cholesterol and triglycerides were associated with obesity.

The confirmed variants are currently under mating to test whether their phenotype has a genetic basis. So far, offspring from five of these mice were investigated. In one case the phenotype (low alanine-aminotransferase activity) proved to be heritable. Two other variants were shown not to be heritable. For the remnant two variants we do not yet have enough offspring to draw a definitive conclusion about heredity.

These data demonstrate the possibility of measuring a large number of parameters from a single blood sample in mice. The recovery of a first genetically confirmed mutant from only a few mice which have passed the entire screening procedure suggests that the clinical chemical screen is a powerful approach to recover mouse mutants with clinically relevant phenotypes.

G12. NEW MOUSE MODELS FOR GENETIC DEAFNESS: A SYSTEMATIC APPROACH TO UNDERSTANDING THE MOLECULAR AND BIOLOGICAL BASIS OF DEAFNESS.

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A new EC-funded programme has been established to generate and characterise new mouse mutants with inner ear defects. A screen for hearing impairment has been added to two large ENU mutagenesis programmes, at Neuherberg and Harwell, and vestibular defects are also being recovered as they lead to circling and head-tossing behaviour. Dominant mutations are presently being collected. A total of 18,000 F1 offspring from mutagenised males have so far been screened, giving 7 mutants with hearing impairment alone and 20 mutants with vestibular defects, some of which are also deaf. Backcrosses are in progress to localise the mutations, and one has been mapped to proximal chromosome 4. The inner ears of three of these ENU-induced mutants with vestibular defects have been studied by paint-filling of whole cleared inner ears to reveal gross malformations, and by scanning electron microscopy to investigate sensory hair cell integrity. In one mutant, the lateral semi-circular canal was truncated, but cochlear hair cells looked normal at 20 days old. In a second mutant, there were no gross malformations, and vestibular hair cells looked normal at 3 days old, but stereocilia bundles in the cochlea were disorganised at 3 and 20 days old. In a third mutant, the posterior and superior semi-circular canals were truncated or constricted, and their ampullae were missing or reduced. This mutant also showed anomalies in organisation of inner and outer hair cells in the cochlea, suggesting that the gene affected in this mutant may affect not only morphogenesis of the inner ear, but also later pattern formation in the organ of Corti. After the initial characterisation, the new mutants will be made available to the research community.

G13. ANALYSIS OF FATTY ACIDS IN MICE USING GC/MS.

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As a part of the functional genomics initiative at the Oak Ridge National Laboratory (ORNL), we are developing a battery of biochemical assays to be utilized in screening the offspring of mutagenized mice. These assays are designed to detect and quantitate a wide variety of important biomolecules, including neurotransmitters, fatty and organic acids, and other biomolecules involved in general metabolic pathways. This will enable us to characterize normal biochemical phenotypes and identify anomalous phenotypes, including non-visible and non-lethal phenotypes that might have been missed in previous studies.

One screening assay is used to identify and quantitate fatty acids in several different mouse tissues, including blood, brain, and liver. Examples of disorders that might be identified by fatty-acid profiling include Refsum disease, Zellweger syndrome, and other diseases caused by partial or complete loss of function of the peroxisome, a ubiquitous mammalian organelle primarily involved in lipid metabolism. This assay can also be used to detect organic acid intermediates in the TCA cycle.

Mouse tissues were treated with base and heated to hydrolyze lipids into their component fatty acids. The hydrolyzed fatty acids were subsequently acidified, extracted, dried, derivatized using N-methyl-N-(tert-butyldimethysilyl)-trifluoroacetamide (MTBSTFA) in pyridine, and analyzed using capillary gas chromatography (GC) using mass spectrometric (MS) detection. Fatty acids containing 8 to 24 carbons, as well as some organic acid metabolites, can be identified and quantitated using this method.

We are able to detect and quantitate a number of fatty acids in brain homogenate, which has allowed us to develop fatty acid profiles for various mouse strains. We will compare and discuss the profiles of several different strains, including BlH, BJR, Bl6/JX, and M24B. The effect of gender and age on these profiles will also be discussed.

G14. A FUNCTIONAL GENETIC ANALYSIS OF THE *brown* DELETION COMPLEX.

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Thirty overlapping deletions flank the brown (now *Tryp1*) locus and allow for a high resolution molecular and functional analysis of 7 - 9 cM on mouse Chr 4. Seven functional units have already been identified within the deletion complex, including brown, whirler (*wi*), depilated (*dep*), brown associated fitness (*baf*), and three lethal complementation groups. Moreover, many genes have been mapped to the deletion region, including several implicated in developmental and/or neurological processes, and others involved in inflammation, wound healing, or the immune response. Together, we are building a sequence-ready physical contig of the entire region, of which 2 Mb of the distal end has already been extensively characterized. In this region, additional transcripts have been identified using exon-trapping, cDNA selection, and human homology mapping.

To carry out a fine structure functional analysis of the region, we are using mutagenesis with N-ethyl-N-nitrosourea (ENU) to isolate mutations that represent single gene functional units. Initial screening has generated candidate alleles of functional units and genes mapped to the region. This pilot experiment has yielded a new mutation in approximately every 25 gametes screened, reflecting the relative gene rich content of the brown deletion region. Our goal is to saturate the region with mutations to generate multiple allelic series, then correlate the mutations with candidate genes. This approach should also generate new models of human diseases encoded by the syntenic region on human Chr 9.

Additional experiments combining ENU mutagenesis with induced and targeted deletions throughout the mouse genome will produce essential data for large-scale expansions of the functional map of the mouse genome in parallel with the human map.

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F. Stassen (D2)
K.P. Steel (A22, A37, B49, B50, E8, G12)
S. Steelman (E18)
L. Stein (A7)
E. Steingrimsson (C13)
V.W. Straub (B31)
R. Straw (A18)
V.A. Street (B5)
C. Streffer (D21)
G.E. Striker (D29)
L.J. Striker (D29)
M.A. Strivens (A18, A26, A46, C9, F4, F14, G10)
R.C. Strom (D41)
L. Stubbs (B47)
T. Suda (E5)
D. K. Sufalko (A14)
R. Suffolk (B45)
Y. Sugahara (C17)
T. Sun (B26)
F. Sundler (C22)
M. Suzuki (C8)
R.T. Swank (B16, B32, B48)
D.A. Swing (B4)

T. Tada (C18)
A. Takada (A32)
T. Takada (B25)
Y. Takahashi (B51)
N. Takakura (E5)
T. Tanaka (B22, C17)
L.M. Tarantino (G2)
A. Tarlton (A17, B14)
M. Tateno (C17)
C. Taya (B25)
V.T. Tchernev (B9)
A. Teale (D23, D24, D25)
U. Teichmann (B8)
B.L. Tempel (B5)
L. Tessarollo (C13)
A. Teubner (F9, G3, G11)
C. Thiel (A41)
S.E. Thomas (E27)
C. Thornton (G4, G5, G10)
T.A. Threat (B20, B26, B37)
A. To (B48)
J.A. Todd (A34)
P. Todorov (B49)
S. Tomita (B11)
M.G. Tordoff (D10)
D.Y. Toy (A19)
Z. Trachtulec (C5)
W. Traut (B52, C25)
J. Treadaway (A49)
D. Threadgill (B38)
R. Trembath (B53)
J. Trent (B8)
L.E. Trepanier (F1, F2, F3, F5, F13)
P.G. Trepanier (F2, F3)
P.J. Trickett (A26, A46)
F. Tronche (C11)
H.T. Tsai (B50)
P.N. Tsichlis (C21)
P. Tsipouras (E22)
P.K. Tucker (E9)
S.K. Turner (D15)
T. Ty (A11)

K. Uchida (D37)
H. Ueda (D8, D39)
Y. Ueda (B35)
M. Uehara (E17)

J. van Arendock (D24)
M. van den Broek (C18)
S. Vandormael (C19)
P.J.J. van Gorp (D32)
H. Van Vugt (A45)
T. van Wezel (D2)
A. Varela (B50)
E. Vasile (E3)
F. Vauti (C7)
M.L. Vetter (E9)
D.L.P. Vetrie (F15)
G. Victorero (C3)
J.M. Vidal (E3)
J. Vilar (D29)
M. Villanueva (A15)
A.J. Villars (A38)
J. Vitale (D6)
L. Vizor (F14, G10)
H. von Melchner (C7)
S. Vriz (B28)

P. Waeltz (A30)
S. Wakana (A48, B25, D37, D40)
Y. Wakabayashi (B25, B51)
M.R. Wallace (B32)
J. Walter (A38)
A. Wang (B41)
D. Q.-H. Wang (D12, D28)
Xi. Wang (B26)
Xu. Wang (B26)
Z. Wang (B29)
A.W. Ward (B11)
S. Warming (C24)
L.L. Washburn (A29)
M. Watahiki (B22, C17)
Y. Watanabe (D40)
D.E. Watkins-Chow (47)
C. Watson (D6)
M.S. Watson (A28)

L.S. Webb (A20, G13)
J.S. Weber (G14)
D. Weichenhan (B52, C25)
C.A. Wells (B53)
T. Wiehe (A4, A18)
J. Weissenbach (A3)
G. Wenske (A27)
C. Werner (C7)
D. West (A20)
P. Weston (A18)
R.A. White (B19)
L.W. Whitehouse (D16)
J.B. Whitney (B38)
L.S. Wicker (A34)
M. Wilda (C26)
B. Wilm (E28)
B. Wielockx (D30)
M. Wiles (A3, C7)
G.W. Williams (A18)
M. Williams (D22)
R.W. Williams (D41)
C.M. Williamson (B53, B54)
M. Williamson (B6)
C.R. Willis (A19)
L. Wilson (C27)
T. Wiltshire (A15)
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M.E. Wines (E4)
J.M. Wingrave (A44)
H. Winking (B52)
T. Wissel (A27)
A.S. Witherden (A24)
E. Wolf (E16, F9, G3, G11)
A. Wong (B29)
P.W. Woollard (A18)
S.F. Wroe (B53, B54)
K. Wunsch (E6)
W. Wurst (C7)

J. Yamate (A32)
H. Yamazoe (A47)
Y. Yamaguchi (B35)
K. Yamamura (C8, E5, E15, E28)
E. Yamoto (D39)
M. Yanagi (A48)
H. Yasui (E14)
T. Yokomine (A31)
Y. Yoneda (B22, C17)
H. Yonekawa (B25, B51, D37)
B. York (A20)
K. Yoshida (C18)
A. Yoshiki (D42)
T. Yoshimoto (D6)
S.G. Younkin (D15)

U. Zechner (D19)
S. Zhang (B34)
W. Zhao (A4, C9)
F. Zheng (D29)
G. Zhou (D41)
H. Zhou (F2, F3)
L. Zhu (A2)
E. Zobeley (A14)
J. Zuo (A49)

Chiroscience R&D is an emerging pharmaceutical company with a clinical focus on autoimmune diseases, osteoporosis and cancer. Our laboratories are located at two sites, one near Seattle, Washington and one near Cambridge, England. Our research programs encompass gene discovery and function, target validation, lead identification, medicinal chemistry and human clinical trials.

Many drugs developed over the next decade will be based on more specific biological models of disease than those currently in use. Our strategy is to use genetics and gene expression information to identify novel targets for drug development. We have programs in human mapping and positional cloning and in mouse genetics aimed at identifying genes that will provide new information about disease mechanisms or susceptibility.

Chiroscience has an active program of scientific collaboration with scientists and research groups from a wide range of universities, private non-profit organizations, and industrial groups. We can bring to these collaborations substantial research capabilities in the areas of genetic mapping, positional cloning and large-scale sequencing, SNP detection, transcription analysis and mutagenesis. We can further the drug discovery process to include screening for small molecule lead identification through to clinical development of drug candidates. We are particularly interested in developing new collaborations focused on the isolation of genes whose products are important in regulating immune function or bone metabolism.