MANIPULATING THE MAMMALIAN GENOME

Organizers: Alan Bernstein, C. Thoams Caskey and A. Dusty Miller

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Manipulating the Mammalian Genome

Keynote Address

D 000 TRANSGENIC MICE AS DISEASE MODELS, Rudolf Jaenisch, En Li, Michael Rudnicki, Hong Wu, Maarten Zijlstra, Whitehead Institute for Biomedical Research, MIT, Cambridge, MA 02142. Three methods have been successfully employed to generate mutations in transgenic mice. These are (i) microinjection of recombinant DNA into the pronucleus, (ii) infection of embryos with retroviruses and (iii) gene transfer into embryonic stem (ES) cells. The expression of mutant genes may lead to dominant phenotypes in cases where the mutant protein interferes with the normal function of the endogenous protein resulting in a dominant negative mutation. Such a phenotype resembling the lethal form of osteogenesis imperfecta has been induced by microinjection of a mutant COL1A1 gene into embryos. Integration of the foreign DNA into the cell genome may also lead to disruption of a gene and to insertional mutations. Because integration by the first two approaches is not predictable, insertional mutations may lead to the isolation of new genes. The resulting mutant phenotypes may be complex and more than one gene may be affected by the insert as will be discussed for the collagen type I mutation in Mov13 mice. DNA transfer into ES cells permits gene targeting by homologous recombination. Targeting frequencies differ by more than three orders of magnitude for individual genes and may be influenced by factors such as gene expression, chromatin conformation or by the presence of recombinogenic sequences. We have derived a mouse strain carrying a targeted mutation in the β2-microglobulin gene and will discuss preliminary observations on the homozygous phenotype.

Developmental Genetics

D 001 MOLECULAR ANALYSIS OF THE MOUSE W/c-Kit LOCUS, Alastair D. Reith, Robert Rottapel, Elizabeth Giddens, Claire Brady, Lesley Forrester, Patrice Dubreuil and Alan Bernstein, Division of Molecular and Developmental Biology, Mt. Sinai Hospital Research Institute, 600 University Avenue, Toronto, M5G 1X5, Canada.
Mutations at the murine dominant white spotting locus (W) have pleiotropic effects on the development of hemopoietic, melanocyte and germ cell lineages as a consequence of intrinsic stem cell defects. Recent work from our group and others has suggested that mutations at the W locus are allelic with the transmembrane receptor tyrosine kinase proto-oncogene c-Kit. We have analysed c-Kit expression and activity in mast cell cultures derived from mice bearing each of nine independent W alleles. Such analyses revealed a deficiency in c-Kit kinase activity in all alleles, the severity of which correlated with the phenotype conferred by each W allele on the whole organism. To understand the molecular basis of these deficiencies we have determined the nucleotide sequence of c-Kit in mice carrying mutant alleles at the W locus. This approach revealed the presence of specific point mutations within the kinase domain of the c-kit polypeptide which result in substitution of single amino acids highly conserved within the protein tyrosine kinase gene family. The location and nature of these substitutions provide direct molecular evidence that W and c-Kit are allelic. The significance of these mutations to the c-Kit signal transduction pathway and to the pleiotropic nature of the defects in W mutant mice will be discussed.

D 002 MOLECULAR GENETICS OF IMPRINTING BY DNA METHYLATION, Wolf Reik and Sarah K. Howlett, Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, United Kingdom.

Developmental failure of parthenogenetic and androgenetic embryos, and the parental origin specific expression of certain genetic traits, are a consequence of genomic imprinting in mammals. Methylation of DNA is possibly one of the components of genomic imprinting. In transgenic mice, parental origin specific methylation patterns have been observed. Genomic modification is susceptible, at least in some transgenic strains, to modifying genes that segregate in inbred strains of mice. Different transgenic inserts seem to be influenced by different genetic factors. Observations on the genetics of factors that influence imprinting suggest that modification can occur both in germ cells and in the embryo after fertilization. We have detected de novo methylation of certain sequences during oogenesis, a finding that could explain why the majority of imprinted transgenes are more methylated when maternally inherited. In contrast to previous reports, we find that the methylation patterns of endogenous sequences of the sperm and egg genome are similar, and that de novo methylation continues after fertilization. In support of our analysis of methylation of specific sequences, we also detect high levels of DNA methylase in the egg and early preimplantation embryo. De novo methylation during oogenesis and after fertilization could contribute to the restricted potency of parthenogenetic cells, and so the methylation of endogenous sequences in fertilized and parthenogenetic embryos is now being compared. Attempts are also being made to study imprinting by methylation of endogenous sequences in later stage mouse embryos and in embryonal tumours in the human.

D 003 MOLECULAR GENETIC ANALYSIS OF CELL LINEAGE DEVELOPMENT **Janet Rossant, Achim Gossler, David Hill, William Skarnes and Alexandra Joyner, Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario, M5G 1X5**

Cell lineage analysis in the early mouse embryo has revealed that only the extraembryonic lineages are set aside as predetermined lineages prior to implantation. A single cell introduced into the embryonic lineage at the blastocyst stage shows no restriction in later tissue fate or position in the developing embryonic axis. However, by the early stages of primitive streak formation, other studies have shown that a fairly precise fate map can be constructed on the epiblast surface. In searching for genes involved in lineage determination, we have, therefore, concentrated on genes whose pattern of expression is restricted to cells of the early established extraembryonic lineages or which show spatially restricted expression around gastrulation. Several genes specifically expressed in the trophectoderm lineage have been isolated by differential screening of cDNA libraries and further characterization of tissue-specific regulation of these genes is underway. To search for genes involved in development of the basic body plan we have utilized lacZ 'enhancer-trap' and 'gene-trap' vectors introduced into embryonic stem (ES) cells. Activation of such constructs by host regulatory elements can result in spatially restricted patterns of lacZ expression in ES-chimeras. We are currently undertaking a large-scale screen of such integration events to identify new genes expressed in specific domains in the early gastrulating embryo. (Funded by MRC and NCI of Canada, NIH).

Generation of Dominant Mutants Using Transgenic Mice

D 004 TUMOR DEVELOPMENT AND PROGRESSION IN v-*jun* TRANSGENIC MICE, Andre C. Schuh[1], Peter K. Vogt[2] and Martin L. Breitman[1], [1]Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, Toronto, CANADA, and [2]Department of Microbiology, USC School of Medicine, Los Angeles, CA.

V-*jun* is the transforming gene of avian sarcoma virus 17. This viral oncogene, as well as its cellular homologues -- c-*jun* and other AP-1 related genes -- encode transactivating DNA-binding proteins which recognize the AP-1 consensus sequence TGACTCA, a response element which confers sensitivity to the tumor-promoting phorbol ester TPA. To investigate the oncogenicity of v-*jun* in multiple cellular lineages, we have generated several lines of transgenic mice carrying v-*jun* driven by the promoter of the ubiquitously-expressed H-2K^{K} class I MHC antigen gene. Animals carrying H-2K/v-*jun* are initially phenotypically normal, but following full-thickness wounding exhibit hypertrophic wound repair which microscopically consists of exuberant, hyperplastic granulation tissue. These lesions are often slowly progressive, and over 2-5 months a fraction gives rise to fibrosarcomas. Mating studies show that within an individual transgenic pedigree, hyperplastic wound repair and its subsequent progression are related to transgene dosage. The reproducibility of this wounding phenomenon makes the H-2K/v-*jun* transgenic mice a useful model for studying tumor development and progression. Not only can the conversion of an abnormality of a benign physiological process to frank malignancy be easily studied, but this progression can be predictably and repeatedly induced. Studies to clarify the nature of v-*jun*-related neoplastic transformation and the role of wound repair in this process are currently underway.

Generation of Mouse Mutants by Gene Disruption or Gene Inhibition

D 005 EMBRYONIC LETHAL MUTATIONS CAUSED BY DNA INSERTION IN TRANSGENIC MICE, Frank Costantini, So-Wun Cheng, James Lee, Christopher Perkins, William Perry III, Glenn Radice and Hyeung Jin Son, Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032. The molecular analysis of mutations affecting mouse embryogenesis represents a powerful method to identify new genes involved in mammalian development. Insertional mutations caused by the integration of foreign DNA in transgenic mice can be readily analyzed, using the transgene as a probe to clone the locus of insertion. We are currently investigating three embryonic lethal mutations caused by transgenic insertions. Embryos homozygous for the ßS12 mutation develop normally to the morula stage of pre-implantation development, but fail to form a blastocyst. Homozygous embryos in line Hß58 develop normally through the early egg cylinder stage and undergo gastrulation, but develop only a rudimentary neural axis and a beating heart, as well as extra-embryonic membranes. Homozygous embryos in line HE46 die at day 9.5 with neural tube abnormalities. DNA from all three mutant loci has been cloned, and a disrupted gene has been identified at the Hß58 locus. The properties of this gene and its relation to the Hß58 mutant phenotype, as well as attempts to identify genes at the other loci, will be discussed.

D 006 RETROVIRAL INSERTIONAL MUTAGENESIS OF THE MOUSE GERMLINE, Nancy A. Jenkins, Leslie F. Lock, Sally E. Spence, Debra J. Gilbert, Deborah A. Swing and Neal G. Copeland, Mammalian Genetics Laboratory, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

The high frequency at which SWR/J-RF/J hybrid mice spontaneously acquire new germline ecotropic proviruses has enabled us to perform a number of studies designed to 1) delineate the mechanism and developmental stage of germline provirus acquisition, 2) evaluate the potential of this approach for viral insertional mutagenesis, 3) explore viral and host genetic factors influencing the frequency of provirus acquisition, and 4) develop a simple method for introducing single copy, expressible proviruses into the mouse germline. The significance of these results with respect to viral insertional mutagenesis and introducing foreign genes into the germline of mice will be discussed. This research was sponsored by the National Cancer Institute, DHHS, under contract NO1-CO-74101 with BRI.

D 007 RETROVIRAL INSERTIONAL MUTATIONS IN EMBRYONIC STEM CELL DERIVED TRANSGENIC MICE, Michael Kuehn, Lisa Cirillo, Vyvienne Attenburrow, Joseph Seow and Philip Iannacone*, Department of Genetics, University of Illinois College of Medicine, Chicago, IL 60612 and *Department of Pathology, Northwestern University School of Medicine, Chicago, IL 60611.

One potential outcome arising from the introduction of DNA into the mouse germ line is an insertional mutation. Compared to conventional methods of mutagenesis, insertional mutagenesis has the powerful advantage that the mutated gene is marked by the exogenous DNA, allowing it to be readily isolated. However, the efficiency of this approach has been too low to allow general application. It has been estimated that the probability of generating an insertional mutation, in the course of deriving a transgenic mouse strain, is 100 fold lower than the probability of producing a mutation using ethylnitrosourea. This is partly due to the fact that transgenic strains produced by the conventional approach usually have only one site of integration of exogenous DNA. One way to improve the efficiency of insertional mutatagenesis would be to increase the number of potential mutagenic events per transgenic animal examined. To this end, tissue culture lines of mouse embryonic stem cells (ES cells) have been infected with a retroviral vector. Multiple retroviral infection of ES cells leads to a high copy number of integrated proviruses, each at a unique chromosomal location. In studies on two trangenic strains derived from such cells, carrying a total of 28 proviruses between them, we have so far identified 4 retroviral integration sites which are associated with recessive developmental lethal mutations. The embryological and molecular analyses of these mutants will be presented. Approaches to further enhance the efficiency of retroviral insertional mutagenesis in ES cell derived transgenic mice will also be presented.

Generation of Targeted Mouse Mutants

D 008 ACTIVITY OF THE MYC-FAMILY OF NUCLEAR ONCOGENES IN NORMAL AND MALIGNANT CELLS. Frederick W. Alt, Tarik Moroy, Averil Ma, Jean Charron, Robert Collum, and Kathryn Zimmerman. The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology, College of Physicians and Surgeons of Columbia University, New York, NY 10032. The myc family of nuclear oncogenes is a dispersed multi-gene family that consists of three well-defined members: c-myc, N-myc, and L-myc. The c-myc gene is expressed in most dividing cells and its deregulated expression by a variety of different mechanisms has been implicated in many different types of tumors; perhaps the most well-characterized is its activation as a result of translocation into the immunoglobulin gene locus in Burkitt's lymphomas. The N-myc and L-myc genes are expressed in a much more restricted set of normal cells, usually only in the very early stages of many different cell lineages. Correspondingly, deregulated expression of these genes has been implicated in a very limited set of naturally-occurring tumors (eg. neuroblastoma and small cell lung carcinoma) and usually only by the mechanism of gene amplification. Amplification of the N-myc gene has been a clinically useful staging and prognostic indicator in neuroblastomas.

Unique structural aspects of individual myc-family genes and their distinct expression patterns during mammalian development suggests that their differential and/or combinatorial expression may play a role in differentiation of cells through particular lineages. We have focused on the potential function of differential myc gene expression during lymphocyte differentiation. Both N- and c-myc are expressed in precursor B and T lymphocytes but only c-myc is expressed after the cells differentiate into mature lymphocytes; L-myc is not expressed in known differentiation stages of this lineage. To study the differential control and functions of myc-family genes, we have generated a variety of transgenic mouse lines that contain introduced N- or L-myc genes expressed under the influence of their own regulatory elements of specifically-targeted and deregulated within the lymphoid lineage. We will discuss how similar deregulation of the N- and L-myc genes differentially predisposes transgenic mouse lines to B or T cell malignancies. In addition, we have disrupted the endogenous N-myc gene in embryonic stem cells and in pre-B cell lines by homologous recombination; these cells have been used for blastocyst injections and resulting chimeric mice are being analyzed for germline transmission. Potential results of the effects of loss of N-myc expression in pre-B cell lines will be discussed.

D 009 **THE MOUSE ENGRAILED - LIKE GENES: FROM DNA SEQUENCES TO GERM LINE MUTATIONS.** **Alexandra L. Joyner, Cairine Logan, Clay Davis, Anna Auerbach, and Janet Rossant, Division of Molecular and Developmental Biology, Mt. Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada, M5G 1X5.**

The two mouse homeo box-containing genes, En-1 and En-2, were identified based on their extensive sequence homology to the Drosophila segmentation gene engrailed. Using RNA in situ hybridization and antibody localization analysis, both genes have been found to be first expressed in spatially restricted patterns that later progress to cell type-specific expression within the same tissues. En-2 is restricted to a mid/hind brain region throughout development whereas En-1 is expressed in cells within the spinal cord, somites and limbs as well as in the mid/hind brain. The regulatory sequences responsible for the early segmental and later cell type-specific expression of EN-2 are being analyzed using lacZ reporter gene constructs introduced into embryonic stem (ES) cells. Chimeric mouse embryos derived from such cell lines can be directly analyzed for appropriate lacZ expression patterns. In order to better understand the function of En-2 in brain development, three mutant ES cell lines have been produced by homologous recombination in which one allele of En-2 is lacking the homeo box coding sequences. One of these cell lines has been used to produce three male chimeras in which the germ line is entirely made up of ES cell-derived cells. Healthy heterozygous offspring carrying the En-2 mutation have been obtained from these males at the expected frequency. A phenotypic analysis of the effect of En-2 mutant homozygosity will now be carried out to determine whether En-2 is essential for normal brain development.
(Funded by MRC and NCI of Canada.

X Chromosome

D 010 STRUCTURE AND FUNCTION OF THE HUMAN Y CHROMOSOME: THE PSEUDOAUTOSOMAL BOUNDARY,

Peter N. Goodfellow and Nathan A. Ellis, Human Molecular Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, U.K.

The Y chromosome is composed of two disparate regions: the pseudoautosomal region (PAR) that is shared between the X and Y chromosomes and Y-specific sequences that include TDF, the testis determining factor. Recombination occurs at a high frequency in the PAR however, recombination in the sex-specific region would lead to break down in the chromosomal basis of sex determination. The pseudoautosomal boundary acts to prevent recombination proceeding from the PAR to the Y-specific region. We have cloned and sequenced the boundary region (1). In the boundary region 4 types of sequence have been found (in the order telomere to centromere): a region of high X-Y chromosome homology; an Alu repeat element inserted on the Y chromosome only; a region of partial X-Y chromosome homology; sex chromosome specific sequences. We have extended these observations by using PCR (polymerase chain reaction) and direct sequencing of the boundary regions from over 60 X and 50 Y chromosomes. We have also sequenced the boundaries from great apes and some old world monkeys. These studies have allowed us to follow the recent evolution of the pseudoautosomal boundary in man. We conclude that sequence discontinuity serves as the pseudoautosomal boundary in these species.

(1) Ellis et al., Nature 337: 81, 1989).

D 011 X-CHROMOSOME INACTIVATION AND REGULATION OF GENE EXPRESSION

Mary F. Lyon, M.R.C. Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.

X-chromosome inactivation in mammals involves regulation of gene expression on a chromosomal scale. The phenomena to be explained include initiation of inactivation in early development from an inactivation centre followed by spreading of the inactivation along the chromosome. Differential methylation of genes on active and inactive X-chromosomes is probably involved in maintaining and stabilising the inactive state. Chromosomal imprinting results in the paternal X being preferentially inactivated in certain cell lineages, but there is no evidence that this imprinting is an essential part of the mechanism. There is growing evidence that some genes may resist inactivation. The spreading inactivation apparently passes through them and inactivates genes beyond. This suggests the existence of sequences specific to the inactivation process but these have not yet been found.

Gene Therapy-I

D 012 RETROVIRAL GENE TRANSFER OF ADENOSINE DEAMINASE INTO HUMAN HEMATOPOIETIC CELLS. Denis Cournoyer, Maurizio Scarpa, Sandra K. Kooyer, Kateri A. Moore, John W. Belmont, and C. Thomas Caskey. Howard Hughes Medical Institute and Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030. We are developing a safe and efficient system of retroviral gene transfer of human ADA into human hematopoietic cells. Inherited deficiency of ADA results in Severe Combined Immunodeficiency (SCID) and serves as a model for the development of gene replacement therapy. The vector ΔN2ADA has been successful in transducing long term expression in mice. Additional vectors have been constructed which have the potential of transducing a tissue specific expression of the enzyme in lymphoid cells. Amphotropic retroviral vectors in GP+envAm-12 with titers of 10^5 to 10^6 are available. *In vitro* and in *vivo* tissue specificity studies are underway. The vector ΔN2ADA has also been used to infect human hematopoietic cells. Virus containing supernatants and cocultivation, with various recombinant growth factors, have been compared for the efficiency of infection of clonogenic progenitors. Following supernatant infection, 5 to 20% of unselected colony forming progenitors contained integrated copy(ies) of the vector as detectable by DNA amplification. Twenty to 100% of those progenitors were infected by cocultivation. With both methods, the best infection efficiency was obtained by infecting the bone marrow cells in presence of growth factors. Low copy number of the provirus were present in supernatant cells from long term bone marrow culture for up to seven weeks post-infection. Transduced ADA expression was detectable by Western blotting following infection of human bone marrow cells derived from two ADA-SCID patients. We are now evaluating the efficiency of infection of long term culture initiating cells and of the primitive human hematopoietic progenitors which appear to be maintained in chimeric human-immunodeficient mice. The results obtained to date indicate that retroviral vectors can successfully transfer and express human ADA into murine and human hematopoietic cells.

D 013 GENETIC MODIFICATION OF SOMATIC CELLS BY USING RETROVIRUS VECTORS, A. Dusty Miller, Michael Kaleko, J. Victor Garcia, William R. A. Osborne* and Theo D. Palmer, Fred Hutchinson Cancer Research Center, Seattle, WA 98104 and *Department of Pediatrics, University of Washington, Seattle, WA 98195.

We have used retrovirus vectors that express a human adenosine deaminase (ADA) cDNA to investigate potential gene therapy involving hematopoietic cells. Vectors containing ADA driven by the SV40 early promoter, human CMV immediate early promoter, or the retroviral LTR were used to infect mouse bone marrow cells. In contrast to results in cultured cells where the SV40 promoter was relatively inactive compared with the other promoters, all vectors produced equivalent amounts of human ADA in mice following long term reconstitution with infected marrow cells. Several animals expressed human ADA at a level equal to mouse ADA in blood at times longer than 4 months after transplantation. To date, infection of marrow used to transplant mice has been performed by using ecotropic retroviral vectors, which cannot infect human cells, and we have shown that amphotropic retroviral vectors, which will infect human cells, can also infect pluripotent bone marrow stem cells in mice. Preselection of infected marrow prior to transplantation significantly improves gene expression following transplantation. We are still losing much of the repopulating ability of mouse marrow during virus infection, and even more during preselection. We have also used the vectors containing ADA to infect normal diploid fibroblasts from rats as a model for gene therapy involving skin cell transplantation. In culture, infection with the vector containing ADA driven by the LTR results in production of human ADA at levels 100-times higher than endogenous rat levels. Transplantation of these cells to rats results in very high levels of human ADA initially, however, human ADA production decreases to undetectable levels by a month after transplantation. The relevance of these experiments to potential human gene therapy will be discussed.

Gene Therapy-II

D 014 ENGRAFTMENT OF NORMAL AND LEUKEMIC HUMAN HEMATOPOIETIC CELLS INTO IMMUNE-DEFICIENT SCID AND BNX MICE, John E. Dick,Suzanne Kamel-Reid, Christian Sirard. Dept of Genetics, Research Institute, Hospital for Sick Children; and Dept. of Medical Genetics, University of Toronto, Toronto, Ontario Canada M5G 1X8. Understanding the process of differentiation and development remains as a major challenge in biology. The blood-forming system of mice and humans consists of a heterogeneous array of cells, ranging from large numbers of differentiated cells with defined function to rare pluripotent stem cells with considerable developmental and proliferative potential. Our understanding of the biology of the human hematopoietic system has suffered relative to that in the mouse because of the lack of an in vivo assay system for human pluripotent stem cells. In addition, progress in understanding human leukemic transformation and progression has been hampered by the lack of experimental in vivo models. We have attempted to develop such in vivo models by transplanting immune-deficient scid and bnx mice with normal and leukemic bone marrow. We have found that human myeloid progenitors rapidly expand in the hematopoietic tissues of bnx mice transplanted with normal human bone marrow and persist there for up to 3 months. The fact that human in vitro progenitors have little if any self-renewal potential and the fact that progenitor cells were continuously produced for at least 3 months suggests that earlier progenitor or stem cells have engrafted these animals. Human bone marrow was infected with a neo vector using high efficiency retrovirus mediated gene transfer techniques and infused into mice; after one month a substantial proportion of the CFU-GM contained the neo gene. It should now be possible to use the retrovirus integration site to follow stem cell clones in the transplanted mice. In addition to normal bone marrow we have engrafted animals with a variety of human lymphoid and myeloid cell lines. In particular we have found that the growth of a human ALL cell line in mice is analogous to the spread of the disease in children. After initial growth in the bone marrow, cells gradually spread to other peripheral tissues and eventually many organs including the lungs, liver, kidney, and brain were massively infiltrated leading to the death of animals by 12 weeks. Bone marrow taken directly from some patients with ALL has also engrafted immune-deficient animals. The establishment of in vivo models for the growth of normal and leukemic cells presents a unique system in which to study numerous biological parameters governing their growth, as well as a system in which to test novel chemotherapeutic agents, biological response modifiers, and immunotherapy techniques for the treatment of human leukemia.

D 015 TREATMENT OF HIV-1 INFECTED hu-PBL-SCID MICE BY CD4 GENE THERAPY, Donald E. Mosier*, R.J. Gulizia*, R. Morgan§, W.F. Anderson§, & S.A. Spector†
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SCID mice reconstituted with human peripheral blood leucocytes (PBL) contain all of the cellular elements of the human immune system and maintain several human immune functions for over one year. These animals, which we term hu-PBL-SCID mice, can be infected with HIV-1 either by injection of cell-free virus or virus-infected T lymphoblasts. HIV-1 can be recovered from the peripheral blood, spleen, and lymph nodes of infected mice by co-cultivation with fresh human T cell blasts. Infection of human cells within the hu-PBL-SCID spleen has been confirmed by *in situ* hybridization and amplification of HIV sequences using the polymerase chain reaction (PCR). HIV infection of hu-PBL-SCID mice causes a suppression of human immune function; *e.g., in vivo* production of human immunoglobulin was suppressed by over 90%. This small animal model for HIV infection has been used to study the expression of human CD4 molecules and their impact on disease progression. Human PBL were infected *in vitro* with a retroviral construct containing genes encoding neomycin resistance and the two N-terminal domains of CD4 as well as the SV40 promoter. These cells were injected before and after G418 selection into hu-PBL-mice previously reconstituted with cells from the same individual. Expression of CD4 molecules in the plasma of these animals has been monitored, and their susceptibility to HIV infection is currently under study. These studies provide an example of the generation of novel animal models for the study of human disease by a combination of xenotransplantation and adoptive gene transfer.

D 016 GENE TRANSFER INTO MURINE HEMATOPOIETIC STEM CELLS AND BONE MARROW STROMAL CELLS, David A. Williams, Bing Lim, Jane F. Apperley, Stuart H. Orkin, David Beier, and Roger Cone, Howard Hughes Medical Institute, Children's Hospital, Boston, MA 02115. The use of recombinant retroviral vectors to transfer genetic sequences into hematopoietic stem cells is one approach to somatic gene therapy. Two limitations of such retroviral vectors are the efficiency of transfer into reconstituting hematopoietic stem cells and the loss of reconstituting ability of hematopoietic stem cells when manipulated *in vitro* during infection and selection. We have investigated the effects of prestimulation of hematopoietic stem cells by growth factors prior to infection on the efficiency of gene transfer. Prestimulation of bone marrow cells in Wehi-3b conditioned media improved the efficiency of gene transfer into CFU-S stem cells. Animals transplanted with bone marrow infected after prestimulation with a simplified retrovirus, Zip PGK ADA, demonstrated long term and stable expression of human adenosine deaminase (ADA) after full hematopoietic reconstitution. In separate experiments, retroviral vectors have been used to transfer the SV40LT antigen sequences in stromal cells making up the hematopoietic microenvironment. Stromal cells expressing LT are immortalized and some support the proliferation of CFU-S stem cells in vitro for up to 4 weeks. Such immortalized stromal cell lines provide an *in vitro* hematopoietic microenvironment which may allow extensive infection and selection of hematopoietic stem cells without loss of reconstituting ability. The use of recombinant retroviral vectors provide a promising approach to correction of human diseases involving bone marrow cells.

Genome Mapping-I

D 017 **TOWARDS A HIGH DEFINITION MAP OF THE CENTRAL SPAN OF THE MOUSE X CHROMOSOME**, Philip AVNER, Luisa DANDOLO, Marie-Christine SIMMLER and Danielle ARNAUD, Unité d'Immunogénétique Humaine, Institut Pasteur, 75015 Paris - France

Increasing numbers of loci have recently been ordered along the mouse X chromosome using both an expanding panel of somatic cell hybrids and progeny from an extensive interspecific mouse cross. This has allowed the definition of the minimum length and number of X-chromosome segments conserved between man and mouse.

Probes bracketing the central span which includes both the X-inactivation center *Xce* and the Tabby locus (*Ta*) equivalent to hypohidrotic ectodermal dysplasia in man, include the X-linked homologue of the *ZfY* candidate gene for the Testis Determining Factor (*Zfx*) , *Ar* the androgen receptor gene and a number of anonymous probes.

Such studies have not only narrowed down one of the évolutionary mouse/human X chromosome rearrangement breakpoints to a 1cM or less region lying between the *Zfx* and Ar genes but also allowed the candidate regions for the X-inactivation center to be highlighted.

Characterisation of additional mouse X chromosome delete material and a further series of somatic cell hybrids using probes in this region has defined an important series of nested overlapping deletes covering the 20 or so centimorgans stretching proximally from the *Dmd* locus and distally to the α galactosidase locus (Ags).

Recent results bearing on our efforts to establish physical short-range map of the candidate regions containing the *Xce* and *Ta* loci by Pulsed-Field gel electrophoresis will be described in this context along with a description of the origins and informativeness of the X-delete material.

D 018 MULTILOCUS MAPPING OF MOUSE CHROMOSOMES USING INTERSPECIFIC MUS CROSSES, Verne M. Chapman, Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, NY 14263

We have mapped 29 genes on the mouse X chromosome in two parallel crosses of Mus species, M. spretus and M. musculus, with the same inbred strain C57BL/6. The map was developed by analyzing the segregation of 12 anchor markers that define more than 75 cM of the X chromosome starting at the centromere and extending to a gene near the X-Y pairing region. An additional series of 17 genes were positioned to specific intergenic regions on the basis of concordant segregation with a panel of 14 backcross progeny that were recombinant for successive interanchor regions. Once a gene was localized to a specific intergenic region, it's relative map order was established by analyzing the segregation of the new gene in all of the backcross progeny that were recombinant in that chromosome region. The relative genetic maps produced in both backcrosses do not differ in gene order and there is no significant variation in the overall recombinational distances. These findings suggest that there are no major differences between Mus species in the relative order of X chromosome genes and that the maps are colinear. An analysis of the cytogenetic location of the mapped genes indicates that the genetic map covers the entire X chromosome from the centromere to band F2-3. The strategy of mapping used in these experiments effectively identifies virtually all of the crossover events on the X chromosome in 200 backcross progeny. On average, this number of progeny will map genes 0.5 cM apart and we should recombine loci 1.5 cM apart 95% of the time. We are interested in determining whether this distance is sufficiently close that we can directly use this information to orient the physical order of genes. Two of the markers in our series, Mcf-2 and NcamL1 fulfill this requirement. Mcf-2 has been physically linked to FIX in the human genome and NcamL1 has been linked to GDX in a similar fashion. In both instances, the genetic map information from the mouse X chromosome is consistent with the order identified in the physical maps. These data suggest that it will be feasible to conduct similar analyses with other sets of genes that have cosegregated in these crosses.

D 019 ESTABLISHMENT AND APPLICATIONS OF A MOLECULAR GENETIC LINKAGE MAP OF THE MOUSE GENOME, N.G. Copeland, A.M. Buchberg, J.D. Ceci, B. Cho, M.B. Cybulski, D.J. Gilbert, M.J. Justice, D.M. Kingsley, L.F. Lock, J.A. Mercer, K.J. Moore, A. Perkins, C.M. Silan, L.D. Siracusa, S.E. Spence, M.C. Strobel, D.A. Swing and N.A. Jenkins, Mammalian Genetics Laboratory, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

Interspecific mouse backcrosses provide a powerful tool for mapping genes on any mouse chromosome. We have used an interspecific mouse backcross to determine the map locations of more than 350 marker loci in the mouse genome. The loci are fairly well distributed among all the autosomes and the X chromosome. The average map resolution is 5-10 centimorgans between loci. We estimate that we now have ~100% probability of determining the map location of any additional molecular probe.

We are using our molecular genetic linkage map of the mouse genome 1) to further understand the genomic organization of the mouse, 2) to identify regions of synteny and linkage conservation between the mouse and human (and other) genomes, 3) to correlate cytogenetic alterations with changes at the molecular level, 4) to determine whether newly identified genes and viral integration sites are homologous to known genes or mutations, 5) to identify molecular loci at or near existing mutations, 6) to identify recessive oncogenes by deletion mapping or reduction to homozygosity of polymorphic alleles, and 7) to establish mouse models for human diseases. The multiple applications of the molecular genetic linkage map will be presented. This research was supported by the National Cancer Institute, DHHS, under contract NO1-CO-74101 with BRI.

D 020 USE OF A TRANSGENIC MOUSE LINE TO STUDY GENOMIC IMPRINTING. D. Solter[1], M. Bucan[1], J. Price[2], J. DeLoia[1], [1]Wistar Institute, Philadelphia, PA 19104; [2]Scripps Clinic, La Jolla, CA 92037.

We have analyzed a transgenic mouse line which appears to have integrated the transgene in an imprinted region of the genome. Transmission of the transgene through the paternal lineage is associated with paw and skull deformities in the offspring; whereas maternal transmission results in phenotypically normal mice. Transcription of the transgenes is not detectable during the time of limb formation, therefore we conclude that the deformity is due to interruption of endogenous sequences. Probes generated from genomic sequences flanking the transgene insertion site were used for chromosomal localization in an interspecies backcross system derived by crossing *Mus musculus* and *Mus spretes*. The integration site maps to mouse chromosome 5 between *Pgm-1* and the proto-oncogene *c-kit*, a likely candidate for the W locus. This region of the genome does not contain any previously identified limb mutations, nor is it an obviously imprinted region, as defined by genetic experiments. Cosmids spanning 45 kbp around the integration site have been isolated and screened for regions which have been conserved between species and to isolate transcriptionally active DNA segments from an 8 1/2 day cDNA library. Additionally, a homeobox domain was detected within 15 kbp of the insertion site, the significance of which is being determined.

Developmental Biology and Genetics

D 100 HOX-7 : A HOMEOBOX LOCUS IN THE MOUSE IMPLICATED IN LOCAL PATTERN FORMATION? Benoît Robert, David Sassoon, Gary Lyons, Jean-Louis Guénet and Margaret Buckingham, Département de Biologie Moléculaire,Institut Pasteur, 28, rue du Dr. Roux, 75724 PARIS Cedex15 France
Expression of the Hox-7.1, a gene that defines a new homeobox locus on mouse chromosome 5, has been studied by *in situ* hybridization. At 11 days *in utero*, transcripts are concentrated in the apical ectodermal ridge (AER) of the limb bud and in the underlying mesoderm, a region essential for the formation of the limb, according to experimental embryology. In collaboration with Pr. John Fallon (University of Wisconsin, Madison), we are investigating whether the expression of Hox-7 is involved in transducing the activity of the AER, using the chick *limbless* mutant to address this question. This mutant forms limb buds at early stages, but is unable to develop AERs and thus limbs. In addition, Hox-7.1 expression is detected in the distal regions of the fronto-nasal and mouth processes. Current evidence suggests that mechanisms similar to those involved in limb formation, involving ecto-mesodermal interactions, are operating to pattern the facial region. Thus, there might be a general function for Hox-7.1 in local pattern formation. Hox-7 maps very close to two mutations, Hammer-toe and Hemimelic extra-toe, which both affect limb formation. We are investigating whether Hox-7.1 is allelic to one of them. Furthermore, we are introducing altered copies of the Hox-7 gene into embryonic stem cells to generate targeted mutations through homologous recombination, in the prospect of reintroducing these mutations into the mouse for a functional analysis of the Hox-7 gene.

D 101 DEVELOPMENT OF CIS-PLATINUM RESISTANCE IN HELA CELLS ACCOMPANIES ENHANCED EXPRESSION OF TRANSFECTED CAT GENE CARRYING CIS-PLATINUM DAMAGE, Chuck C.-K. Chao[1] and Sue Lin-Chao[2], [1]Tumor Biology Laboratory, Department of Biochemistry, Chang Gung Medical College, Taoyuan, Taiwan 33333 and [2]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, U.S.A
Acquired resistance of HeLa cells to cis-platinum (CP) was established by stepwise selection. Expression of CP damaged plasmid DNA carrying chloramphenicol acetyltransferase (CAT) gene after transfection into cells was measured. Cells resistant to CP showed greater CAT activity than parental cells in such a way that fold resistance accompanies enhanced enzymatic activity. Enhanced expression of damaged CAT gene was not observed in revertants derived from CP resistant cells. Addition of aphidicolin, an inhibitor for DNA polymerase alpha, effectively blocked enhanced CAT activity and acquired resistance in CP resistant cells. The results suggest that cellular ability in processing DNA damage is a potential mechanism for CP resistance of cells. Experiments looking for proteins that interact with CP damaged DNA are currently in progress.

D 102 TRANSACTIVATION BY THE HIV TAT REGULATORY PROTEIN IN TRANSGENIC MICE, Ted Choi, Marc Alizon, and Rudolf Jaenisch. Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142.

Transgenic mouse lines carrying the HIV tat regulatory protein driven by a human histone H4 promoter were generated. By northern analysis, one of these lines (F3tat-9) showed high level expression of tat in every tissue examined. At eight months of age, these mice evidenced no disease phenotype. Mice bearing an HIV-LTR-CAT transgene were bred to the F3tat-9 line. Transactivation of LTR-CAT by tat was seen in all tissues except the liver, where no LTR expression was detectable. The degree of transactivation varied from 2-fold in the heart to over 20-fold in the submaxillary gland. To determine whether the different transactivation levels reflect a mechanistic difference or are simply due to variations in the fraction of LTR-CAT expressing cell types also expressing tat, we are separating cells, as well as establishing cell lines, from various tissues of these mice.

D 103 **INTEGRIN EXPRESSION IN EMBRYONIC STEM CELLS,** Helen M. Cooper, Fiorella Altruda and Vito Quaranta, Research Institute of Scripps Clinic, La Jolla CA 92037. Integrins are transmembrane receptors for matrix components, mediating adhesion and migration of cells. Their role in development is thought to be critical. Little is known however about integrin expression in early embryonic stages in mammals. For instance, no data are available on integrin expression in embryonic stem (ES) cells. By immunofluorescence, we have found that murine ES cells CCE express a single integrin heterodimer ($\alpha_E\beta_4$), which previous studies in our laboratory showed to be epithelial cell-specific (e.g., it is found on keratinocytes, gut epithelium, cytotrophoblast). Distinctive characteristics of this epithelial integrin are that it is asymmetrically located on the cell surface, i.e., in areas of contact with substrate, and that it functions as a major receptor for the basal lamina, likely by binding to laminin. Interestingly, in addition to epithelial integrin we also detected laminin on CCE cells, as punctated surface immunofluorescence. This raises the possibility that an interaction between epithelial integrin and laminin may contribute to early topographic organization of the mouse embryo, and makes the corresponding genes obvious candidates for targeting. Progress on these experiments will be presented.

D 104 **GENE TRANSFER TO HEMOPOIETIC STEM CELLS WITH LYMPHO-MYELOID REPOPULATING ABILITY AND THEIR MAINTENANCE IN MURINE LONG TERM BONE MARROW CULTURES,** C. Fraser, C.J. Eaves, S. Szilvassy and R.K. Humphries, Terry Fox Laboratory, Cancer Control Agency of British Columbia and University of British Columbia, Vancouver, B.C. Canada.

As an approach to gaining further insights into hemopoietic stem cell regulation, we have begun to combine retroviral infection and the long-term culture system. Marrow from 5-fluorouracil treated male mice was co-cultivated with or exposed to supernatants from helper-free psi-2 cells expressing high titres of a recombinant virus carrying the neo gene under conditions previously shown to allow efficient infection of lympho-myeloid stem cells. Infected marrow cells ($3x10^6$/culture) were seeded onto pre-established irradiated female feeders and 4 weeks later assayed for retrovirally marked day 12 CFU-S and repopulating cells. Viral integration was detected in 45% of day 12 CFU-S with no common bands seen in CFU-S recovered from the same cultures. Contributions to hemopoiesis by LTC derived cells was assessed in 5 female recipient mice 45 days post transplantation. In all mice greater than 50% of marrow, spleen and thymus were donor derived as determined by Southern blot analysis with a Y specific probe. In 1 mouse no evidence of reconstitution with virally marked cells was detected and in a second mouse evidence of multiply marked clones was detectable only in the spleen. Strikingly, however, in 3 mice a single viral integration band unique to each mouse was present in DNA of spleen, marrow, and thymus at readily detectable levels. These results provide the first direct evidence that stem cells with lymphoid and myeloid repopulating potential can be maintained in culture and thus serve as a starting point for characterizing factors controlling their growth.

D 105 **MAINTENANCE OF A METHYLATION-FREE ISLAND IN TRANSGENIC MICE,** **Glenn Gundersen, Frank Larsen, Hege Munch and Hans Prydz, Research Institute for Internal Medicine, University of Oslo, 0027 Oslo 1, Norway.** The dinucleotide CpG has several interesting features in the DNA of vertebrates. It occurs at about $^1/_5$ of the expected frequency - probably as a consequence of a second feature; methylation. Between 70% and 90% of all genomic CpG's in vertebrates are methylated at the 5-position on the cytosine ring, and is thought to be an evolutionary response to the expanding genome in terms of gene regulation and region silencing. Our main interest concerns a third feature(a dual paradox from those mentioned above)namely; clusters of non-methylated CpG's, also known as methylation-free islands (MFI's). Typically MFI's, ranging from 500 to 2500 base pairs, are shown to comprise the proximal end of genes - probably affecting their expression. We wish to unveil the mechanism of how MFI's are maintained through mouse development, and have therefore generated transgenic mice with a 1.35 Kb MFI fragment from the murine Thy-1.1 gene. Analysis of the methylation state of the transgene in various tissues from F_3-homozygous individuals are currently under study. Furthermore, theoretical secondary structure analysis combined with <u>in vitro</u> methylation and Band-shift assays are consistent with the emerging hypothesis of structurally protected CpG's in these non-methylated regions.

D 106 RESCUE OF THE ALBINO PHENOTYPE BY THE TYROSINASE GENE IN TRANSGENIC MICE, Edith Hummler, Friedrich Beermann, Siegfried Ruppert, Franz X. Bosch, Günter Müller and Günther Schütz, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, FRG

The c-locus of the mouse is thought to encode tyrosinase the key enzyme for melanin synthesis in melanocytes of the skin and retina. We have previously isolated a mouse tyrosinase cDNA by screening mouse B16 melanoma cDNA libraries (Ruppert et.al., 1988; EMBO 7, pp. 2715-2722). In a functional assay, this cDNA clone confers tyrosinase activity to a cell line which expresses no specialized functions for melanin synthesis (Müller et.al., 1988; EMBO 7, pp. 2723-2730). To prove that the isolated DNA represents the genetically well characterized c-locus, a minigene containing the tyrosinase cDNA and flanking genomic DNA sequences of the chinchilla allele was constructed and microinjected into fertilized oocytes of an albino mouse strain (NMRI). Five transgenic lines were established which showed pigmentation in skin and eyes. Northern analyses and in situ hybridization suggest that the expression of the transgene is restricted to melanocytes. Thus we demonstrate that the albino mutation (c/c) can be rescued by introduction and expression of a functional tyrosinase gene.

D 107 RECONSTITUTION OF MUTANT W MICE WITH CULTURED NEURAL CREST DERIVED MELANOBLASTS: AN IN VITRO SYSTEM FOR THE STUDY OF MELANOBLAST DIFFERENTIATION, Dennis Huszar and Rudolf Jaenisch, Whitehead Institute, Cambridge, MA 02142

Melanoblasts, the precursors of melanocytes, are derived from the neural crest, a transient structure formed at the site of closure of the neural tube in vertebrate embryos. Neural crest cells migrate extensively through the embryo giving rise to a variety of differentiated cell types, including melanocytes and neurons.

In a previous study (Jaenisch, Nature 318, 181-183, 1985) we demonstrated that crest cells derived from the explanted neural tubes of C57Bl/6J embryos could participate in normal development and contribute to hair pigmentation following in utero injection into mid-gestation Balb/c embryos. We have now tested mice carrying mutations at the W (dominant white spotting) locus for their competence as recipients of melanoblasts from cultured C57Bl/6J crest cells. Mutations at the W locus have pleiotropic effects, one of which is a lack of hair pigmentation due to an absence of melanocytes in the skin of affected mice. On the other hand albino mice, such as Balb/c, contain a full complement of melanocytes in the hair follicles; they lack pigment due to a deficiency in tyrosinase, the key enzyme in melanin synthesis. We find that mutant W embryos are capable of far more extensive reconstitution with exogenous melanoblasts than are Balb/c, likely due to a lack of competition with endogenous melanocytes in the mutant mice. We typically observe pigmentation in approximately 40% of the W offspring, in some cases corresponding to coverage of as much as 70% of the skin. Experiments are underway to use this system to examine the role of the c-kit proto-oncogene in melanoblast differentiation.

D 108 PROTHROMBIN GENE TRANSCRIPTION RATE DURING GESTATION: FETAL LAMB MODEL, C.T. Kisker, A.L. Olson, S. Perlman, Department of Pediatrics, University of Iowa, Iowa City, IA 52242.

Prothrombin is known to be developmentally regulated. In fetal and newborn lambs, the appearance of prothrombin activity increases with gestational age. We have shown that changes in prothrombin activity are reflected in both prothrombin antigen levels and prothrombin mRNA levels in fetal and newborn lambs (J Lab Clin Med 112:407-412, 1988). Prothrombin gene expression thus occurs at either transcription or post-transcriptional processing of the gene transcript. To test if transcription of the prothrombin gene is developmentally regulated, nuclear run-on transcription assays were performed on sheep liver nuclei isolated from fetuses (130 days gestation) and newborn lambs (9-11 days). ^{32}P-labeled transcriptions were hybridized to prothrombin and β-actin (internal standard) cDNAs which had been immobilized on nylon filters. Autoradiograms of the hybridized filters were quantitated using densitometry. Prothrombin transcription rate was determined as the ratio of prothrombin transcripts to β-actin transcripts at each age. The transcription ratio for fetal nuclei was 0.57 $\pm$ 0.20 (mean $\pm$ S.D., n=3). For newborn nuclei the ratio was 0.40 $\pm$ 0.08 (n=4). The differences were not significant (p=.180). The findings indicate that during gestation prothrombin gene expression is not primarily regulated at the level of transcription but more likely at the level of post-transcriptional processing and stability of the gene transcripts.

D 109 A NOVEL METHOD FOR RETROVIRAL TITERING USING THE POLYMERASE CHAIN REACTION (PCR), Daniel Kuebbing[1], Richard Morgan[2], W. French Anderson[2], and Kathy Stambaugh[1], [1]Genetic Therapy, Inc., Gaithersburg, MD 20878 and [2]Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892. Determination of the concentration of retroviral vector in culture supernatants is routinely performed by assay of the ability to transduce a drug resistance gene to appropriate target cells. This procedure is tedious, slow, and the variance of the assay is relatively large. The polymerase chain reaction (PCR) has been investigated for use as a quantitative method to determine retroviral titers. Viral RNA is detected after partial purification from culture supernatants. The relationship between RNA content, as determined by PCR, and biological activity has also been studied.

D 110 ANALYSIS OF PERI-IMPLANTATION STAGE AND GASTRULATION STAGE INSERTIONAL MUTATIONS, Elizabeth Lacy, Elayne Bornslaeger, Michele Blum, Kathy Signorelli, Yale Jen, Nils Lonberg, and Willie Mark, Molecular Biology Program, Sloan-Kettering Institute, New York, NY 10021

We have identified two transgenic mouse lines that carry recessive prenatal lethal insertional mutations, line 4 and line T81-3. Line 4 was generated by the integration on chromosome 3 of a tandem array of 6 to 8 copies of a bacteriophage recombinant, λRβG2. Mice homozygous for the λRβG2 insertion become arrested in their development at implantation on the 5th day of gestation. The site of integration has been cloned from both the line 4 and wild type C57BL/6J genomes, and an analysis of these clones revealed a 23 kb deletion of host DNA at the mutant locus. To date we have reproducibly detected a 3.5 kb transcript in kidney with two independent probes prepared from one of the two junctions between the λRβG2 insert and flanking host DNA. A recently completed tertiary screen of an adult mouse kidney cDNA library identified 31 potential clones of the 3.5 kb transcript. These cDNA clones are currently being characterized by restriction enzyme mapping and nucleotide sequencing. The transgenic line T81-3 was generated by the integration of a tandem array of 3 to 5 copies of a human CD8α construct. Mice homozygous for the CD8 insertion appear to become arrested in their development at 6.5 to 7.5 days p.c. An examination of abnormal embryos generated in a line T81-3 intercross has identified a distinct mutant phenotype consisting of an underdeveloped embryonic ectoderm and an "overdeveloped" extraembryonic ectoderm. Experiments are now in progress to further characterize the mutant embryos by histological sectioning and to clone the mutant and wild type loci.

D 111 RAPID VARIATION IN MAMMALIAN MITOCHONDRIAL DNA: CONSTRUCTION OF HETEROPLASMIC MICE BY SINGLE-CELL EMBRYO FUSION, Philip Laipis, Ted Choi, Rudolf Jaenisch, Kirsten Fischer-Lindahl, and Bruce Loveland, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610; Whitehead Institute for Biomedical Research, Cambridge, MA 02142; Howard Hughes Medical Institute, Dallas, TX 75235.

The details of the mechanism which transfers mammalian mitochondrial DNA (mtDNA) from mother to offspring and the constraints which this mechanism places on the origin of mitochondrial genetic diversity are currently unknown. The frequent appearance of mtDNA sequence polymorphisms within a species suggests only a small subset of the maternal mitochondria are transferred to the new embryo. Passage through this bottleneck could permit the rapid appearance of new mtDNA genotypes in the population. We have tested this model directly by constructing mice with two mtDNA genotypes and examining the inheritance of mtDNAs from founder females. The techniques of McGrath and Solter (Science (1983) 220:1300-1302) were used to transfer oocyte cytoplasm from eggs of C57BL/6 mice to NMRI/BOM mice and vice-versa. Four heteroplasmic mice containing 30-60% mitochondria derived from the donor strain were obtained. Initial results show that the transferred mitochondria function normally and are transferred by females (but not by males) to offspring. Variations in the levels of the two mtDNA species are seen in the F1 and F2 generations. These animals support for the model described above. They are the first step in the development of methods to construct animals carrying in vitro mutated mtDNAs.

D 112 EVIDENCE FOR REPRESSOR BINDING ACTIVITY SPECIFIC FOR A MOLONEY MURINE LEUKEMIA VIRUS SILENCER THAT INHIBITS EXPRESSION IN EMBRYONAL CARCINOMA CELLS, Tatjana Loh, Laura Sievert, Richard Scott, E.I. DuPont de Nemours Co, Inc. Central Research and Development, Wilmington, DE 19880. A negative regulatory element spanning the tRNA primer binding site (PBS) of Moloney murine leukemia virus (M-MuLV) represses M-MuLV expression specifically in embryonal carcinoma (EC) cells. By base-pair mutagenesis, we have precisely defined the element as the 18 bp of the tRNA PBS. Since restriction of expression from the M-MuLV promoter is independent of the position and orientation of the tRNA PBS element, we characterize it as a silencer sequence that is preferentially active in mouse EC cells. A DNA binding activity specific for the M-MuLV tRNA PBS sequence has been detected in Exonuclease III protection assays using crude nuclear extracts. Binding is strongly correlated with repression in EC cells: mutations within the tRNA PBS that relieve repression disrupt binding activity, and nuclear extracts from permissive, differentiated EC cell cultures show reduced binding activity for the tRNA PBS sequence. These results indicate the presence of a stem cell-specific repressor that extinguishes retroviral expression via the silencer element.

D 113 PROMOTER EFFECTS ON THE EXPRESSION OF GENES IN A CONSISTENT RETROVIRAL BACKBONE, Jeanne R. McLachlin[1], Michael J. Kadan[1], Donna Williams[1], Edmund Wonilowitz[1], Lydia Gould[1], MaryBeth Daucher[1], W. French Anderson[2], and Martin A. Eglitis[1], [1]Genetic Therapy, Inc., Gaithersburg, MD 20878 and [2]Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892. Retrovirus vectors, packaged using packaging cell lines, provide an efficient means of introducing foreign DNA sequences into mammalian cells. To facilitate identification and quantitation of potential high titer vector producing clones, a selectable marker gene is often included in the vector. Current vector designs have the selectable marker regulated either by the viral LTR, or by some internal promoter. Since packaged vector results from transcripts generated from the proviral LTR, we wanted to determine if vector titer was an equally accurate measure of total infectious particles when the marker used for titering was driven off an internal promoter instead of the LTR. In addition, we wanted to evaluate the effect of different internal promoters on the apparent vector titer. To this end, we have constructed a series of vectors containing both the bacterial neomycin resistance gene (neo^R) and the bacterial β-galactosidase (β-gal) genes. For these studies, we designed a retroviral vector backbone which permitted precise and consistent placement of genes within the backbone. In one set of vectors, neo^R is regulated by the LTR while β-gal is regulated by an internal promoter. A second set of vectors was constructed as the exact reciprocal, thereby reducing the chance that differences in levels of gene expression might be the result of subtle positional effects. Titers were evaluated in two ways: 1) by comparing neo^R to β-gal titer in the same backbone and 2) by comparing one backbone arrangement with its reciprocal. Different promoters were also analyzed for their effect on titer based on the internal gene.

D 114 TRANSGENIC RATS: NEW MODELS FOR HYPERTENSION J. Mullins, J. Peters and D.Ganten, German Institute for High Blood Pressure Research and Dept.of Pharmacology, University of Heidelberg, INF 366, D-6900 Heidelberg, FRG.
Primary hypertension is considered a polygenic inherited disorder but to date the nature of the genes involved is unknown. Transgenic techniques are now widely used but are performed almost exclusively in the mouse. The reasons for this are that the technique was first established in this species, it is genetically well characterised and its short generation time and ease of handling facilitate experimentation. However, the mouse has limitations in certain areas of research such as hypertension and the cardiovascular system. To overcome these limitations and generate new models for hypertension we decided to establish transgenic rats as a new tool for research in these areas.
We have now succeeded in generating transgenic rats by introducing the mouse Ren-2 gene into the genome of the rat using transgenic techniques. Two independant lines of transgenic rats have been established and those animals which possess the mouse gene exhibit extreme hypertension (systolic pressure 180-260). The segregation of the hypertensive phenotype with the presence of the transgene, in independant lines, indicates that expression of the mouse renin gene is resonsible for the hypertension. Interestingly these animals do not have abnormally high levels of renin in their plasma, and have significantly lower levels of renin in their kidneys, suggesting that over-expression of renin in the kidney does not account for the hypertension. These animals are a possible model for normal or low renin hypertension and high tissue renin. We are presently determining the underlying cause of their hypertension.

D 115 CARDIAC ACTIN GENE EXPRESSION IN SOMITES IN THE EARLY MOUSE EMBRYO: CELL LINEAGE ANALYSIS WITH THE lacZ GENE REPORTER IN TRANSGENIC MICE, Marie-Odile Ott, Yvan Lallemand, Serge Alonso and Margaret Buckingham, Department of Molecular Biology, Pasteur Institute, 75724 Paris Cedex 15, France.

Cardiac actin is one of the first genes, characteristic of an adult tissue to be expressed during embryogenesis. By in situ hybridization, transcripts are detectable in the cardiac tube from 7.5 days and in the myotomal cells of the somites which will give rise to skeletal muscle from 8.5 days, before myotomal muscle formation and accumulation of other contractile protein such as the myosins (Sassoon et al., Development 104:155 (1988)). In order to look more closely at the first stages of myogenesis in the embryo, we have constructed transgenic mice carrying the proximal promoter region of the cardiac actin gene (-320/+120) fused to the bacterial lacZ marker gene. Five founders (Fo) have been obtained and the F1 are currently under analysis. The proximal promoter region of the cardiac actin gene used, has been demonstrated to be functional in cell transfection experiments (Daubas et al., NAR 16:1251 (1988)) although analysis of cardiac actin gene expression in BALB/c mice which have a modified cardiac actin gene locus suggests that further upstream sequences may be necessary for optimal expression in the adult heart (Garner et al., Dev. Biol. 134:236 (1989)). The extent of ß-galactosidase expression in the transgenics will be informative in this context, and expression of the marker gene should permit us to address the questions of when cells expressing cardiac actin first arise in the embryo, and of whether cells which migrate from the somites, to found skeletal muscle masses other than the adjacent myotomal muscle, also stain positively for ß-galactosidase and can therefore be followed using this marker.

D 116 SPERM VECTORS: EFFECTS OF PHENOL RED AND DNASE ON BINDING OF DNA TO SPERM. Kimball O. Pomeroy, Carlisle P. Landel and Glen A. Evans, Molecular Genetics Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92037.

Production of transgenic mice by pronuclear injection is an important technique in the analysis of mammalian development. Lavitrano, et al. (Cell 1989; 57: 717-723) described a novel method for the production of transgenic mice in which oocytes were fertilized with DNA-coated sperm. Failure to repeat this experiment led us to examine various parameters affecting the binding, and possible internalization, of DNA by mouse sperm. We compared the binding of DNA to sperm using media with and without phenol red and determined the binding kinetics of DNA to sperm. Cauda epididymal sperm from C57BL/6 mice were capacitated in Whittingham's medium with or without phenol red (.0025%) for 30 min, followed by 30 min exposure to 0.5 µg of radiolabelled DNA (pRSV-luciferase). Sperm were washed and centrifuged twice in medium without phenol red and binding of DNA to sperm was determined by Cerenkov counting. In four replicates, phenol red had no effect on the binding of DNA to sperm. To determine if DNA was internalized by the sperm, DNA-coated sperm were exposed to DNase (250 µg/ml) for 12 min. Bound DNA decreased from 1.25 ng/10^5 sperm to 0.18 ng/10^5 sperm. In all experiments, maximal binding of DNA to sperm occurred within 15 to 30 min. DNA-coated sperm were used to fertilize mouse oocytes *in vitro* and were transferred into pseudopregnant recipients. Offspring (n=21) were assayed for integrated transgenes by the polymerase chain reaction and dot blot hybridization. No transgenic mice were detected from any of the sperm vector experiments. We conclude: 1) phenol red does not inhibit binding of DNA to sperm, 2) substantial amounts of DNA may not be internalized by sperm, and 3) our failure to produce transgenic mice using this technique is not due to failure of the sperm to bind DNA.

D 117 AN ANALYSIS OF TRANSLATIONAL REGULATION OF TESTIS-SPECIFIC *Pgk-2* USING TRANSGENIC MICE, Murray O. Robinson, Jesus Del Mazo[+] and Melvin I. Simon, Division of Biology, California Institue of Technology, Pasadena, CA 91125 and [+]Centro de Investigaciones Biologicas, Madrid, Spain.

The gene for testis-specific phosphoglycerate kinase (*Pgk-2*) is thought to be regulated at both the transcriptional and translational level. In transgenic mice containing the human *Pgk-2* gene, we have shown previously that the transgene is transcriptionally regulated in a manner similar to the endogenous *Pgk-2* gene, and that sequences 5' to the coding region of human *Pgk-2* are sufficient to confer this transcriptional control upon CAT and luciferase reporter genes.

To analyze translational regulation of the human *Pgk-2* transgene, we have now monitored the developmental appearance of human and mouse PGK-2 protein by Western blots and cellulose acetate isozyme electrophoresis. Accumulation of human and mouse PGK-2 protein is similar during testis development, suggesting that the transgene is also translationally regulated.

Furthermore, *in situ* localization of *Pgk-2*/CAT and murine *Pgk-2* RNAs using a dispersed testis cell technique demonstrates that both of these messages accumulate in the cytoplasm with peak expression in late pachytene cells. Immunofluorescence techniques, however, detect murine PGK-2 protein only in elongated spermatids and residual bodies, whereas luciferase protein can be found at a high level in pachytene cells. This suggests that the *Pgk-2*/luciferase and likely the *Pgk-2*/CAT genes, which share 3' untranslated sequences from SV40 T antigen, are missing the sequences required for translational control. We are currently testing transgenic mice containing other regions of the human *Pgk-2* gene for sequence elements that control translational regulation.

D 118 EXPRESSION OF A MUTANT ALPHA 1 (I) COLLAGEN GENE AFFECTS PATTERN FORMATION IN TRANSGENIC CHIMERIC MICE, Michael A. Rudnicki, Hong Wu, John F. Bateman, and Rudolf Jaenisch, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142. Type I collagen, a major component of the extracellular matrix, is a multimeric complex of three chains. This multimeric feature of collagen facilitates the genetic analysis of collagen structure and function since the introduction and expression of a mutant collagen may disrupt normal collagen structure in a dominant fashion. The DNA sequence encoding the procollagen cleavage site at the carboxyl terminus of the mouse alpha 1 (I) collagen protein was altered by site-directed mutagenesis to produce a change in the amino acid sequence at this cleavage site. *In vitro* analysis suggests that this carboxyl terminus mutation (designated CT), appears to either block or impede cleavage of the carboxyl terminal peptide from the alpha 1 (I) procollagen protein. The CT mutant was transfected into embryo-derived stem (ES) cells and several independent lines were derived. The expression of the transgene was analyzed during *in vitro* differentiation of the ES cell lines to determine in which lines was the transgene subject to appropriate regulation. Preliminary results suggest that high expression of the CT transgene and/or high contribution of the ES cell to the chimera, produce a dominant lethal phenotype characterized by vascular rupture shortly after birth. In contrast, lower levels of CT expression and ES cell contribution often result in viable chimeras. Many of these chimeric mice exhibit supernumary digits on one or more paws. These results suggest a hitherto unsuspected role for extracellular matrix during limb bud pattern formation.

D 119 **ANALYSIS OF MYOGENESIS BY GERM LINE TRANSFORMATION APPROACHES,** **Moshe Shani, Institute of Animal Science, The Volcani Center, Bet Dagan 50250, ISRAEL.**

Myogenesis is characterized by the transition of proliferating mononucleated myoblasts to post-mitotic multinucleated fibers. This is associated with the activation of a large bettery of genes, whose products are involved in the formation of the contractile apparatus and the provision of energy for muscle contraction. To define control mechanisms involved in the activation of the myogenic program, we employ transgenic mice and multipotent early embryonic stem (ES) cells.

The skeletal muscle actin gene is expressed in skeletal and cardiac muscles, although the myogenic determination genes MyoD1, myogenin and myf-5 are expressed exclusively in skeletal muscle. To determine whether the same regulatory elements are functional in both striated muscles, transgeic mice carrying different CAT constructs were analyzed. The results indicate that 140 bp of the 5' flanking region of the actin gene are sufficient to confer preferential expression in both striated muscles, and that high level of specific expression is dependent on the presence of at least the entire first intron.

MoyD1 was the first identified gene involved in muscle determination and differentiation. To determine whether MyoD1 can stably convert any cell type to the myogenic program, we are analyzing the consequences of a constitutive expression of MyoD1 in ES cells and their differentiated derivatives. Preliminary data indicate that the expression of MyoD1 in ES cells does not alter their multipotentiality, and that only a subset of differentiated cell types are responsive to MoyD1, indicating that MyoD1 is probably not the mater regulatory gene for skeletal myogenesis.

D 120 TISSUE AND SPECIES-SPECIFIC REGULATION OF THE THY-1 GENE. J. Silver, M. Koyama P.G. Chesa, W.J. Rettig, and J.W. Gordon. North Shore University Hospital/Cornell University Medical College, Manhasset, New York; Memorial Sloan-Kettering Cancer Center, New York, New York; Mount Sinai School of Medicine, New York, New York. Thy-1 is a differentiation marker which has been conserved throughout evolution and is expressed in a number of different tissues including thymocytes and neurons. One of its more intriguing characteristics is the marked interspecies variation in its expression profile. For example, in mice Thy-1 is expressed on thymocytes and peripheral T cells while in rats it is absent from peripheral T cells. In man, expression in the lymphoid system is restricted even more in that Thy-1 is absent from both thymocytes and peripheral T cells even though it is still expressed in the brain. Altogether, there are nine tissues where the expression of Thy-1 differs in mouse and man. In an attempt to delineate the molecular mechanisms responsible for these species differences in expression profile, we have constructed transgenic mice containing human, mouse or chimeric mouse-human Thy-1 genes. The results of these studies indicate that expression of Thy-1 in various tissues is regulated by multiple and independent cis-acting DNA elements associated with the Thy-1 gene and that the differences in expression between mouse and man are due to differences in the number and organization of these cis-acting DNA elements. These studies also demonstrate that Thy-1 expression in thymocytes and peripheral T cells is controlled by separate elements as is Thy-1 expression in the molecular and granular layers of the cerebellum. Furthermore, some of these cis-acting elements appear to be located more than 2 kb upstream of the promoter.

D 121 RETRIEVAL OF THE FLANKING SEQUENCES FROM THE *LEGLESS* MICE

Gurparkash Singh, Dorothy Supp, John McNeish and S. Steve Potter I.D.R., Children's Hospital Research Foundation, Cincinnati, OH 45229.

Legless, a homozygous recessive insertional mutant obtained by integration of the pHT1-1 transgene in the murine genome, lacks hindlimb structures distal to the femur and exhibits malformed forelimbs. In addition to the limb deformities these mutants are viable for a very short period after birth and show severe brain dysmorphology especially in the anterior structures of the cerebrum, including the olfactory lobes. The heterozygous mice of this line, however, bear a normal phenotype and live a normal life span. These observations suggest that pHT1-1 transgene has disrupted an endogenous gene that plays an important role in early embryogenesis. The retrieval and characterization of the flanking sequences will enable identification of this gene. To this end we have employed a strategy that involves circularization of the end pieces containing the flanking sequence, amplifying these fragments by polymerase chain reaction, cloning in a suitable vector and analyzing the clones. Furthermore, field inversion gel electrophoretic analysis will be done to delineate the structural organization of the transgene concatamer and the juxtaposed murine genomic sequences.

D 122 EFFECTS OF MUTANT KERATIN PROTEIN EXPRESSION ON DIFFERENTIATING AGGREGATES OF F9 EMBRYONAL CARCINOMA CELLS, Katrina T. Trevor, Center for Molecular Biology, Wayne State University, MI 48201

The biological role of simple epithelial keratins in the morphogenesis of polarized epithelium has yet to be defined. The mouse keratins Endo A and Endo B are expressed in all simple epithelial cell types of the adult and are the first intermediate filaments expressed during early embryogenesis. Endo A/Endo B filaments are detected in the 8 cell stage embryo and are observed in the trophectoderm and later in extraembryonic visceral and parietal endoderm. Differentiating aggregates of F9 embryonal carcinoma cells serve as a useful three-dimensional model of processes of early embryonic epithelial cell differentiation to visceral or parietal endoderm cell types. Expression of a truncated Endo B protein, encoded by a transfected plasmid construct, causes a dramatic collapse of the keratin network in a stably transfected F9 clone induced to differentiate in monolayer. Preliminary studies demonstrate that cell aggregates of this clone readily differentiate to parietal endoderm-like cells. However, aggregates induced to differentiate to visceral endoderm appear to shed the outer visceral endoderm-like cell layer, suggesting that keratin filaments play a fundamental role in maintaining the integrity of embryonic visceral endoderm.

D 123 CONSTRUCTION OF MUTATIONS IN THE α1(I) PROCOLLAGEN GENE WHICH RESULT IN RESISTANCE TO COLLAGENASE CLEAVAGE. Hong Wu, Alex Stacey, Rudolf Jaenisch and Stephen Krane. Whitehead Institute for Biological Research, Department of Biology, Massachusetts Institute of Technology, cambridge, MA 02142

Collagenase is the only enzyme which can cleave undenatured type I, II and III collagen helices at neutral pH. The cleavage site is located at a specific locus between Gly-Ile or Gly-Leu bonds (residues 775-776). In order to understand the mechanism of collagenolysis, mutations in the α1(I) procollagen gene have been constructed. These mutants were transfected into homozygous Mov13 cell lines in which the expression of α1(I) collagen is completely blocked by retrovirus insertion, although a normal amount of α2(I) mRNA is synthesized. Triple-helical type I collagen containing substitution of Pro for Ile (776) was not cleaved by human rheumatoid synovial collagenase, whereas that containing substitution of Met for Ile (776) was cleaved. Double substitutions of Pro for Gln (774) and Ala (777) also made type I collagen resistance to the cleavage. Interestingly, the α2(I) chains in these collagen molecules were also resistant indicating the presence of normal α1(I) chains are critical for cleavage of the α2(I) chains in the type I heterotrimer. Our observation indicates that the properties of local hydrophobicity and structure are important for the collagenase activity, and all three chains in the type I heterotrimer must contain the appropriate sequences for the enzyme to work. The above mutants are currently been used to produce transgenic mice. This experiment will reveal how interfering with collagen turnover affects embryonic development as well as organogenesis.

D 124 ANALYSIS OF THE METHYLATION STATUS OF pBR322 SEQUENCES IN TRANSGENIC MICE: THE IDENTIFICATION OF A SEQUENCE(S) WHICH DIRECT METHYLATION, K. Yager, G.P. Singh, J. McNeish, and S. Potter, Children's Hospital Research Foundation, Cincinnati, OH 45229

In *legless*, an insertional mutation in transgenic mice, the PHT 1-1 transgene is hypermethylated and refractory to standard cloning techniques. We, and others, have found that the inclusion of bacterial vector sequences in the transgene contruct often leads to lower levels of transgene expression or to nonexpression.[1] The inclusion of vector sequences has been correlated with an increased level of cytosine methylation as monitored by restriction enzyme cleavage with isoschizomers sensitive and insensitive to recognition site methylation.[2] To further investigate the cause of the hypermethylation in PHT1-1, the vector sequences included in PHT1-1 have been subdivided into three fragments and transgenic mice have been generated. Control lines containing the entire pBR322 plasmid have been generated and each line demonstrates hypermethylation. Fragment 1 (1425 base pairs, EcoR1 to Ava 1 in pBR322) exhibits a similar methylation pattern to that produced by the entire plasmid in four transgenic lines. Fragment 2 (783bp, Pst I to Hind III in pBR322) does not exhibit detectable methylation in two transgenic lines. Fragment 3 (1369bp, Nru1 to Pvu1 from pBR322 including a deletion of bp1092 to 2485), has been used to generate 3 transgenic lines and methylation patterns are currently being analyzed. In addition, Fragment 1 is being further subdivided to more closely identify a specific sequence which directs methylation of itself and perhaps of surrounding genomic sequences. Many lines of evidence implicate methylation as a process involved in controlling development and more specifically in controlling gene expression. Identifying a specific sequence which directs cytosine methylation may elucidate a mechanism for epigenetic switches in development.

[1]Townes, T.M., et. al., 1985. EMBO Vol 4, no. 7, pp. 1715-1723.
[2]Humphries, R.K., et. al., 1985. Am. J. Hum. Genet. 37:295-310.

D 125 CYTOKINE GENE EXPRESSION IN MOUSE EMBRYONIC DEVELOPMENT, Choy-Pik Chiu, Rich Murray and Frank Lee, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. Lymphokines such as interleukins 1-7 and hemopoietic growth factors such as granulocyte and macrophage colony stimulating factors (G-, M-CSF) play an important role in regulating the immune response in adult animals. To investigate their role in the development of the hemopoietic system, we have analyzed the pattern of lymphokine gene expression during mouse embryonic development. Using polymerase chain reactions (PCR), we detected the presence of mRNA for interleukin 6 (IL-6) and leukemia inhibitory factor (LIF) but not IL-3, IL-7 or GM-CSF in d. 3.5 blastocysts. IL-6 bioactivity was also detectable in cultured blastocysts. It is not clear whether IL-6 and LIF are produced by trophoblasts or the inner cell mass of the blastocysts. However, PCR analysis of embryonic stem cell (ES) RNA indicated the presence of both lymphokines. These results suggest that the inner cell mass, from which ES cells were derived, may be a source of IL-6 and LIF. Preliminary experiments suggested the expression of IL-6 and LIF mRNA in d. 8.5 embryos as well. Our finding that lymphokines are expressed in early embryos implies a role for them in embryonic development. We are currently targeting lymphokine genes in ES cells by homologous recombination and hope to produce mutant animals to study the effects of lesions in the lymphokine genes on hemopoiesis in vivo.

Insertional and Targeted Mutations

D 200 GENE CORRECTION BY HOMOLOGOUS RECOMBINATION AT THE DELETED aprt LOCUS IN CHO CELLS, Yasuaki Aratani*, Yukio Shiomi*, Dai Ayusawa#, Takeshi Seno$ and Hideki Koyama*, *Kihara Institute for Biological Research, Yokohama City University, Yokohama, 232, Japan; # Institute of Applied Microbiology, University of Tokyo, Tokyo, 113, Japan; $ National Institute of Genetics, Mishima, 411, Japan.

Targeted homologous recombination is a powerful tool for permitting precise gene correction, site-specific gene modification or targeted gene disruption. Using a hemizygous adenine phosphoribosyltransferase (aprt) deficient Chinese hamster ovary (CHO) cell line, which lacks most of the 5th exon of the gene, as a recipient, we studied the correction of deletion mutation by homologous recombination. Transfection with a plasmid carrying a deletion encompassing over the 1st and 2nd exons of the aprt gene yielded $APRT^+$ recombinants at a frequency of up to $3X10^{-4}$ survivors. This frequency is ten to a hundred times higher than the values reported. Linearlization of the introduced DNA by cutting at the homologous site between the deletions reduced the appearance of recombinants. Southern blot analysis indicated that the recombinants carried the wild-type aprt gene and suggested that they should result from either gene conversion or double cross-over events between chromosomal and introduced DNA sequences.

D 201 **GERM LINE TRANSMISSION AND EXPRESSION OF A CORRECTED HPRT GENE PRODUCED BY GENE TARGETING IN EMBRYONIC STEM CELLS.** Alan R. Clarke, Simon Thompson, Angela M. Pow, Martin L. Hooper, and David W. Melton. Department of Pathology, Medical School, Edinburgh University, Edinburgh, UK.

The deletion mutation in the HPRT deficient mouse embryonic stem (ES) cell line E14TG2a has been corrected by gene targeting. The presence of plasmid sequences in the correcting vector DNA did not affect the frequency of correctants. Cells from one corrected clone have been introduced into mouse blastocysts, and germline transmission of the ES cell-derived corrected gene has the same pattern of expression as the wild-type gene, with the characteristic elevated level of expression in brain tissue. Hence, we have demonstrated the feasibility of introducing targeted modifications into the mouse germ line by homologous recombination in ES cells. We now hope to extend this technology to other genes.

D 202 **INTRODUCTION OF NULL MUTATIONS INTO THE MURINE C-ABL AND C-MOS PROTO-ONCOGENES BY HOMOLOGOUS RECOMBINATION IN EMBRYONIC STEM CELLS.** William H. Colledge and Martin J. Evans. Department of Genetics, University of Cambridge, Downing St., Cambridge, CB2 3EH, U.K.

We are interested in understanding the role(s) of the c-*abl* and c-*mos* proto-oncogenes in mammalian development. It is hoped that an insight into the normal function(s) of these genes will clarify their role in cell transformation. The c-*abl* locus encodes two major proteins which differ in their N-termini. The role of each variant in unknown but it has been postulated that each protein may have a specific function in embryonic development. Activation of the c-*abl* gene by chromosomal translocations is observed in two human leukaemias, CML and ALL.

The c-*mos* gene is transcriptionally repressed in most adult tissues but high level expression is observed in haploid germ cells. The c-*mos* gene probably plays a role during meiotic division and may also have a function in macrophage differentiation.

We are investigating the functions of the c-*abl* and c-*mos* proto-oncogenes by generating mutant mice carrying null alleles of these genes. To generate these mice we are using various types of construct designed to enable efficient selection of ES cells in which the target gene has been modified by homologous recombination. Targetted ES cells can be used to generate chimaeric mice with germ line transmission of the modified gene from which transgenic null mutants can be bred.

A progress report will be presented.

D 203 CREATING CONDITIONAL DEVELOPMENTAL MUTATIONS WITH ANTISENSE RNA, R. P. Erickson, A. Bevilacqua, R. Loch-Caruso, Dept. of Human Genetics and Ped., Univ. of MI Sch. of Med., and Dept. of Environmental and Industrial Health, Sch. of Public Health, Ann Arbor, MI 48109-0618. Although homologous gene replacement in embryonic stem cells with subsequent placement in embryos is rapidly being developed to generate developmental mutants, most current strategies will generate null mutations. Antisense RNA's driven by inducible and developmental/tissue specific promoters offer an approach to generating conditional mutations which we are currently exploring. We have found that the mammalian preimplantation embryo will tolerate up to 20 pg of RNA injected into the cytoplasm without harmful effects on development. It this is a capped RNA transcript, it persists for reasonably long periods of time. We have injected a number of sense and antisense RNAs and shown that antisense RNA to several genes results in inhibition of expression of their products, sometimes with marked effects on the morphology of development. Specifically, antisense RNA to a cDNA for β-glucuronidase can inhibit expression up to 75%. The inhibition can reach 90% when the antisense RNA is to a 5' fragment including the start codon. Embryos injected with a transgene using the metallathionine promoter to drive expression of an antisense RNA to β-glucuronidase cDNA also show up to 90% inhibition. We have attempted to inhibit β-glucuronidase expression with oligodeoxynucleotides but we have not yet obtained inhibition or significant uptake. Antisense RNA to a liver gap junction (GJ) protein cDNA which includes 5' transcribed material has dramatic effects on preimplantation development. These effects were related to inhibition of GJ communication by the use of dye markers; rhodamine dextran as a non-GJ permeable marker and Lucifer yellow, a GJ permeable dye.

D 204 ANTI-SENSE RNA CAN MODULATE SURFACE DR7 EXPRESSION IN TRANSFECTED L5.4 CELLS. Elizabeth H. Field and Todd Rouse, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242. To test the hypothesis that class II MHC molecules mediate autoimmunity one needs to develop congenic animals that do not express the MHC molecule of interest. One approach would be to use anti-sense RNA and gene therapy to turn off translation of the targeted MHC gene. To determine the feasibility of this approach, we examined whether anti-sense RNA could alter surface expression of DR7 in L5.4 cells (a DR7 expressing mouse fibroblast clone; Klohe, et al., J. Immunol. 141:2158, 1988). We subcloned fragments of the DR7β chain cDNA into pSRα296 expression vector in the anti-sense orientation, and co-transfected these plasmids with neo^R cDNA into parental L5.4 cells using $CaPO_4$ mediated DNA transfer. After three weeks selection with HAT plus G418 cells were stained with monoclonal anti-class II antibodies and analyzed by FACS. DR7 surface expression was reduced 75% in cells transfected with the 5′ DR7β cDNA fragment (TR-SRαC⁻). Cells transfected with the 3′ fragment DR7β cDNA (TR-SRαB⁻) showed <10% reduction of surface DR7 compared to parental cells. Northern blot analysis of TR-SRαC⁻ total RNA and densitometry showed an anti-sense:sense RNA ratio of 3:1. There was no decrease in the level of endogenous DR7α or DR7β chain RNA. By 12 weeks after transfection the level of surface DR7 protein in TR-SRαC⁻ cells had normalized and no anti-sense RNA could be detected, suggesting that the anti-sense DR7β plasmid was incorporated into the L5.4 genome in an unstable fashion. The results that suggest anti-sense DR7β RNA decreases DR7 surface expression in TR-SRαC⁻ cells, possibly by affecting DRβ7 protein through a post-transcriptional mechanism.

D 205 **INSERTIONAL MUTAGENESIS OF HUMAN CELLS USING RETROVIRUS SHUTTLE VECTORS** ANDREW J. GROSOVSKY[1,2], LESLIE S. HASEGAWA[1], CAROL M. BOYD[1] AND ADONIS SKANDALIS[2], [1]University of California, Riverside, California 92521 and [2]York University, Toronto, Ontario M3J 1P3

In order to characterize parameters of insertional mutagenesis in human cells, the human B lymphoblastoid cell line TK6 was exposed to infection with the retrovirus shuttle vector pZipNeo for periods of 12 to 72 hours. A population containing stably integrated provirus was obtained by selecting for G418 resistance, encoded by the *neo* gene in the vector genome. The infection efficiency varied with infection period, ranging up to 3% in cells exposed for 72 hours. The provirus copy number was surprisingly low (1 to 3 proviruses per $G418^R$ cell) and unlike infection efficiency, did not increase with longer infection periods.

An infection period dependant induction of mutations was observed at several endogenous selectable markers. Following a 72 hour infection period, induction ranged from approximately 5 fold at *hprt* and *tk*, to 30 fold at *aprt*. In approximately 1/2 of the APRT⁻ mutants, the *aprt* locus was shown to be disrupted by a retroviral shuttle vector insertion. However, no intragenic proviral integrations were observed for the other APRT⁻ mutants despite the 30 fold increase in mutation frequency. Similarly, no proviral integrations were recovered within the the *tk* or *hprt* loci. Nevertheless, the mutational spectra at both *hprt* and *tk* is clearly distinguished from spontaneous mutagenesis as reflected in a significant under-representation of genomic rearrangements and loss of heterozygosity events. Therefore, retroviral insertional mutagenesis in this system appears not to be limited to integration within the target locus. Rather, the majority of induced mutations may be attributable to extinction of gene expression created by a retrovirus insertion event outside of the locus at risk.

D 206 ***C-JUN* EXPRESSION IN ES CELLS AND TRANSGENIC MICE.** F. Hilberg and E. F. Wagner. I.M.P., Dr. Bohr Gasse 7, A-1030 Wien, Austria.

The nuclear proto-oncogene *c-jun*, the first proto-oncogene shown to be a transcription activator, codes for an AP-1 binding activity and was furthermore shown to be identical to the *fos* associated protein p39.

To investigate the role of *c-jun* during mouse development we are generating mouse strains with either ectopic expression of the *c-jun* gene (gain of function) and o r with the inactivated *c-jun* gene (loss of function).

In order to study the consequences of overexpression of *c-jun in vivo* we placed the coding region of both the mouse and the human gene under the control of strong constitutively expressed enhancer/promoter sequences, such as the human-ß-actin promoter, the H2 promoter, the viral RSV and PCMV promoters (in collaboration with R. Bravo, M. Karin and U. Rüther). The different *c-jun* constructs were introduced into fertilized eggs by DNA-microinjection and several transgenic lines were obtained. Introduction of these constructs into ES cells by electroporation led to the isolation of high expressing ES cell clones. High *c-jun* expression did neither alter the growth parameters nor did it change the undifferentiated ES cell morphology. These ES clones are being used for blastocyst injection and the generation of chimaeric mice. Expression analysis from both the transgenic mice and the chimaeras will be reported.

In a complementary approach we are attempting to inactivate the *c-jun* gene by gene targeting through homologous recombination in order to analyse the role of *JUN* in mice lacking one member of the *jun* gene family. Progress from this experiment will be discussed.

D 207 HOMOLOGOUS RECOMBINATION IN TISSUE-SPECIFIC GENES IN ES CELLS; MODELS FOR PHYSIOLOGICAL INVESTIGATIONS INTO GENE FUNCTION, Randall S. Johnson,[1,3] Morgan Sheng,[2] Michael E. Greenberg,[2] Richard D. Kolodner,[1,3] Virginia E. Papaioannou,[4] and Bruce M. Spiegelman[1,3], Departments of Biological Chemistry and Molecular Pharmacology,[1] and Microbiology,[2] Harvard Medical School, the Dana-Farber Cancer Institute,[3] and the Department of Pathology, Tufts University[4], Boston, MA 02115

We have used murine embryonic stem cells (ES cells) in an attempt to study the function of certain genes which mayplay important roles in tissue-specific cellular and systemic physiology. We have made constructions using the sequences encoding the c-fos, adipsin, aP2, and alpha-tumor necrosis factor genes and used these constructions to alter their endogenous counterparts via homologous recombination. The constructs were designed to make the genes dysfunctional, by disrupting either transcription or translation. Homologous recombinants in these genes were isolated in ES cells using a variation of the double selection method of Mansour, Thomas, and Capecchi. While we saw rates of homologous recombination over random integration (neomycin resistance) of between 10^{-3} and 10^{-5} in these transfections, which were consistent from construct to construct, we saw no correlation between these rates and the levels of expression of the genes in ES cells. c-fos, which is expressed at a low level in ES cells (as assayed by Northern blot) shows recombination at a rate of 2×10^{-4}; aP2 and adipsin, adipocyte-specific genes, have no detectable message on Northerns and show homologous recombination rates of 5×10^{-3} and 5×10^{-4}, respectively. We have used these ES cell lines to make chimeric mice in both outbred (CD-1) and inbred (C57/Bl6) strains. We have generated chimeras with the c-fos, adipsin, and aP2 recombinant ES cell lines, and are attempting at present to determine whether there has been a germ line contribution in any of the chimeras.

D 208 ANALYSIS OF SHORT TERM AND LONG TERM EXPRESSION OF GENES TRANSDUCED BY RETROVIRAL VECTORS USING FLUORESCENCE ACTIVATED CELL SORTING, Michael J. Kadan[1], Susan Schmitt[2], Robert Wersto[2], Robert C. Moen[1], W. French Anderson[3] and Martin A. Eglitis[1], [1]Genetic Therapy, Inc., Gaithersburg, MD, 20878, [2]Molecular Oncology, Inc., Gaithersburg, MD 20878, and [3]Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892. Retroviruses have proven extremely useful as vectors for gene transfer into mammalian cells. Much is still unknown, however, about the initial cellular attachment and internalization of virus and subsequent expression of viral genes. Recombinant retroviruses with selectable markers for both drug resistance and a readily assayable enzyme activity make it possible to determine levels of viral gene expression at various times and compare this to the frequency of stable, long term integration events. We have exposed murine fibroblasts (NIH 3T3) to a recombinant ecotropic retrovirus containing the β-galactosidase (β-gal) and neomycin resistance (neo^R) genes. Using a fluorescinated β-gal substrate, we analyzed cells by fluorescence activated cell sorting for percent transduction and level of viral gene expression. Our preliminary results indicate that 48 hr after exposure to the virus at a multiplicity of infection (MOI) of approximately 0.1 (based on titers by G418 selection), greater than 90% of cells were positive for β-gal expression. Furthermore, as the MOI was increased towards 1.0, the level of β-gal expression approached 100%, possibly indicating multiple viral copies per cell. These results suggest that ecotropic retrovirus may transduce NIH 3T3 cells transiently at a much higher efficiency than estimated from the number of stable, G418-resistant integrants, and that much of the viral gene expression observed at 48 hr after infection may be transient in nature.

D 209 TARGETED MODIFICATION OF THE MURINE UROKINASE TYPE PLASMINOGEN ACTIVATOR GENE IN EMBRYONIC STEM CELLS, S. Kaur, M. Mucenski, K. McLain, S. Potter and J. Degen, Children's Hospital Research Foundation, Cincinnati, OH 45229

Urokinase type plasminogen activator (uPA) catalyzes the conversion of plasminogen to the active serine protease plasmin. A strong correlation has been observed between presence of uPA activity and cellular processes involving tissue remodelling and cell migration events such as trophoblast implantation and neural crest cell migration. This has led to the suggestion that uPA through generation of localized proteolytic activity may be an important regulator of these cellular processes, although the exact role is unknown. In order to determine the role of uPA in development, we are attempting to functionally inactivate this gene by homologous recombination in embryonic stem (ES) cells. A targeting vector designed according to the positive-negative selection strategy of Mansour et al (Nature 336, 348-352, 1988) has been generated. The vector contains 13-14 kb of target homology, with the neo^R gene inserted into the uPA gene coding region and with the HSV-TK gene placed distant to the homology region. D-3 ES cells were electroporated with the targeting vector and double transformants ($G418^R$ and $gancyclovir^R$) were selected for further analysis. Pools of selected cells were screened by polymerase chain reaction (PCR) and positive pools containing the targeted cells have been identified. Currently, efforts are in progress to isolate the targeted ES cell clones from the positive pools. Preliminary results as determined by PCR analysis indicate successful isolation of individual targeted ES clones. Confirmatory Southern blot analysis of these clones is currently in progress. Once confirmed, the targeted clones will be used to generate chimeric mice from which uPA null mutants will be generated.

D 210 INACTIVATION OF THE MURINE CD2 AND CD4 GENES BY HOMOLOGOUS RECOMBINATION IN EMBRYONIC STEM CELLS,
Nigel Killeen and Dan R. Littman, Department of Microbiology and Immunology and Howard Hughes Medical Institute, University of California, San Francisco, CA 94143.
CD2 and CD4 are non-polymorphic T lymphocyte surface glycoproteins whose respective ligands are thought to be the LFA-3 and MHC Class II molecules respectively. A number of *in vitro* experiments suggest that the interaction between these molecules and their ligands is integral to the process of T cell activation in response to antigen. To test this hypothesis and to clearly define the *in vivo* function of CD2 and CD4, we are developing mouse strains which do not express these molecules. To achieve this we have electroporated embryonic stem cell lines with mutant genomic CD2 or CD4 constructs and screened the transfectants for inactivation of the chromosomal loci by homologous recombination. The gancyclovir selection protocol of Mansour et al (1988; *Nature* **336**, 348-352) was employed and cell lines carrying the desired mutant alleles were identified by the polymerase chain reaction and verified by Southern blots. These cells are now being introduced into inbred mouse blastocysts in order to produce germline chimaeric animals.

D 211 FUNCTIONAL ANALYSIS OF THE MOUSE *Dmd* GENE.
Maconochie, M.K., Greenfield, A.J., Fairbrother, U.L. and Brown, S.D.M. St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK.
Dystrophin dysfunction in humans is associated with Duchenne muscular dystrophy. The human cDNA sequence has been used in protein and DNA sequence homology searches, and these define four functional domains. The human cDNA has been used to identify the mouse homologue *Dmd*. We have initiated a project to investigate the *in vivo* function of specific regions of *Dmd* and have already isolated genomic sequences. Site-directed mutations of such sequences will be targeted to the endogenous locus in embryonic stem cells. This will be accomplished by utilising the *neo* and *Hprt* genes in the homologous integration vector pSB1 as selectable markers for the detection of homologous recombinants in a positive/negative selection system. pSB1 has already proved successful for one stage selection, following trial transformations involving pSB1 alone and selecting for $G418^R$. Homologous recombinants will be used to generate somatic and/or germline chimaeras by microinjection of blastocysts followed by reimplantation in pseudopregnant females. Any domain-specific effects can then be analysed in chimaeric mice and transgenics if germline chimaeras are obtained.

D 212 TARGETED DISRUPTION OF THE RAS GTPASE ACTIVATING PROTEIN (GAP) GENE IN NIH3T3 CELLS.
Mark S. Marshall, Wendy S. Hill and Richard A.F. Dixon. Merck Sharp and Dohme Research Laboratories, West Point, PA. 19486. The RAS GTPase activating protein (GAP) is the only mammalian protein yet identified which interacts with Ras. GAP has been shown to physically associate with oncogenic Ras proteins in vitro and has been proposed to be either the biological target of Ras or a primary regulator of Ras activity. Since ras oncogenes are routinely identified in about 30% of all human tumors, it is of significant interest to determine what role, if any, GAP may play in human cancer. To address this question we are generating null alleles of the murine GAP gene in NIH3T3 cells. The primary gene disruption was obtained by homologous recombination of a prematurely terminated coding exon into a chromosomal GAP gene. The cell line containing this disruption is being cloned and examined for reduced GAP activity and gross changes in cellular physiology. To ascertain whether GAP is an essential gene, a secondary gene disruption will be made in the primary GAP disruption strain. If GAP is indeed the Ras biological target, no secondary disruptions will be obtainable. In contrast, if GAP activity is directed as a Ras regulator, a secondary disruption should result in unregulated cell growth.

D 213 PURIFICATION AND CHARACTERIZATION OF MAMMALIAN RECOMBINASE, Yuji Mishina*, Reiko P. Abe*#, Toshibumi Shimokawa*, Akiko Hata-Tanaka#, Hideki Koyama*, *Kihara Institute for Biological Research, Yokohama City University, Yokohama, 232, Japan; # Biochemical Research Labolatory, Morinaga Milk Industry, Co.Ltd., Zama, 228, Japan.

For the purpose to develop a efficient gene targeting system, we had purified and characterized mammalian recombinase. Two methods were applied for homologous recombination assay; interplasmid recombination assay with two kinds of pSV2neo derivatives (rec activity), and strand transfer assay. When cell-free extract prepared from mouse mammary carcinoma FM3A cells was fractionated on a DEAE-Sephacel column, two peaks of rec activity were eluted with 200mM and 350mM NaCl. Strand transfer activity was co-eluted at 200mM NaCl. Both activities eluted with 200mM NaCl were adsorbed on a Z-DNA Sephadex column and eluted with 400mM NaCl. The eluted strand transfer activity was homology dependent, but ATP- and magnesium-independent, and free from ligase activity. This activity was resistant from inhibitors of mammalian topoisomerase I and II.

D 214 INACTIVATION OF THE I-Aβ GENE IN ES CELLS. Mark W. Moore, Jörg Zwirner, Michael J. Bevan, and George Widera, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Several strategies have been devised to identify the rare ES cell in which a nonselectable gene has been altered by homologous recombination. Using a positive/negative double selection procedure, Mansour *et al* were able to inactivate an oncogene, int-2, which is expressed at very low levels in ES cells. A sensitive RNase protection assay showed no transcription of the Aβ gene, the target gene in our experiments, in the ES cells used. No successful alteration by homologous recombination of a silent gene in ES cells has been reported thus far. Because of the expected low frequency of this event, we applied very stringent enrichment procedures for the targeted integration event.

The Aβ gene in the embryonic stem cell line CCE is the target of inactivation by using a sequence replacement strategy employing positive and negative selection for the integration event by homologous recombination. As a targeting sequence, we used a 1.6 kb NdeI-BglII fragment of the Aβ (b) gene. In order to disrupt the exon encoding the β 1 domain of this gene and to provide a selectable marker, we inserted a modified neomycin resistance gene into the BstEII site of this plasmid.

To further increase the proportion of homologous recombination events, we have integrated two HSV1-TK genes as negative selectable markers on both ends. Cells which have integrated the targeting sequence in a homologous recombination event will survive G418 treatment and gancyclovir application because they lack the HSV1-TK gene. DNA was introduced by electroporation and 35 clones survived selection, 5 of which were shown to have undergone homologous recombination.

D 215 INACTIVATION OF THE C-FOS GENE IN ES CELLS BY MICROINJECTION AND HOMOLOGOUS RECOMBINATION, Catherine E. Ovitt and Ulrich Rüther, European Molecular Biology Laboratory, Heidelberg, West Germany

In order to examine the role of c-fos protein in mouse development, we are attempting to inactivate the c-fos gene in embryonic stem (ES) cells by homologous recombination. To optimize the efficiency of recombination, constructs with increasing lengths of sequence homology to c-fos (2.1 to 26 Kb) have been isolated, in which the c-fos coding region has been interrupted by an oligonucleotide encoding a stop codon and frameshift mutation. These constructs have been introduced into 3T3 and ES cells by microinjection. As c-fos is normally expressed at a low level in ES cells, we have induced c-fos transcription by serum stimulation at the time of microinjection. Detection of potential recombination events by the Polymerase Chain Reaction (PCR) has been accomplished on pools of microinjected cells using specific oligonucleotide primers. The numbers of PCR positives obtained from microinjection in both 3T3 and ES cells suggest that induction of c-fos expression increases the frequency of recombination at the c-fos gene.

D 216 TRANSGENIC MICE EXPRESSING HAMSTER PRION PROTEIN PRODUCE SPECIES-SPECIFIC SCRAPIE INFECTIVITY AND AMYLOID PLAQUES, S. B. Prusiner, M. Scott, D. Foster, C. Mirenda, D. Serban, F. Coufal, M. Wälchli, M. Torchia, D. Groth, G. Carlson, S. J. DeArmond and D. Westaway, UCSF, San Francisco, CA 94143, McLaughlin Institute, Great Falls, MT 59401
The insert of a cosmid clone containing the Syrian hamster (Ha) prion protein (PrP) gene was microinjected into fertilized mouse (Mo) (C57BL6xSJL)F_2 embryos. Three transgenic (Tg) founder mice were identified and mated to produce lines designated 69, 71 and 81. All 3 lines expressed the cellular isoform of HaPrP (PrP^C) in their brains at a concentration similar to that found in hamsters. Inoculation of Tg 81 mice with Ha prions caused scrapie in ~75 days; hamsters inoculated with Ha prions had the same incubation period while non-Tg control mice failed to develop scrapie after >500 days. Inoculation of Tg 71 mice with Ha prions caused scrapie in ~150 days, similar to the time required for developing scrapie after inoculation with Mo prions. Tg 71 and 81 mice inoculated with Ha prions exhibited spongiform degeneration and reactive astrocytic gliosis while Tg 81 mice also showed HaPrP amyloid plaques characteristic of hamster scrapie. Both Tg 71 and 81 mice produced the hamster scrapie PrP isoform (PrP^{Sc}) in their brains after inoculation with Ha prions. The brains of Tg 81 mice with scrapie were found to contain ~10^9 ID_{50} units/g of Ha prions based on Ha bioassays. Tg mice carrying a HaPrP minigene failed to express $HaPrP^C$ and had scrapie incubation times after inoculation with Ha prions similar to those observed for non-Tg controls. These findings argue that the PrP gene modulates scrapie susceptibility, incubation times and neuropathology. Our studies are the first demonstration of the synthesis of infectious scrapie prions programmed by a recombinant DNA molecule.

D 217 PRODUCTION OF HUMAN HEMOGLOBIN S ANTILLES IN TRANSGENIC MICE E.Rubin, E.Spangler, P.Curtin, E.Witowska, B.Lubin, and S. Clift. Lawrence Berkeley Laboratory, Berkeley, CA, Oakland Children's Hospital , Oakland CA and U.C.S.F., S.F., CA.
The development of a mouse model for sickle cell anemia should facilitate studies on the pathophysiology and treatment of this disorder. We previously introduced a human β^S -globin gene into mice (Am. J. Hum. Genet. 42:585-591, 1988). Red cells from these animals did not sickle and we suspect that this was due to the low level of expression of human β^S as well as the absence of human α-globin chains. To improve our effort to develop a phenotypic model for sickle cell anemia we have genetically engineered mice to synthesize human Hb S Antilles. Due its decreased solubility, this sickling β chain varient causes clinical disease even as a trait. In constructing these animals the erythroid specific DNAse I hypersensitive site II was linked to a 1.5 kb Pst I fragment containing the human α 2-globin gene and separately to a 5 kb Bgl II fragment containing the β^S Antilles gene. Six transgenic animals derived from embryos injected simultaneously with both constructs contained between 2-30 copies of both the human α and the human β^S Antilles transgene. Of these animals, four synthesize a hemoglobin species that migrates upon I.E.F. to where we predict Hb S Antilles should focus. Reverse phase HPLC analysis of globin chains from these animals show that of the total α and β chains present, the human chains comprise between 4-15% of each. Tryptic digests of isolated globin chains by Fast Atom Bombardment Mass Spectrometry reveal peptides consistent with human α and β^S Antilles globin chains. Taking advantage of the unique properties of Hb S Antilles, we hope to develop an animal model where in vivo sickling will occur under physiological conditions.

D 218 ANALYSIS OF MURINE *Nmyc* FUNCTION BY IN SITU HYBRIDIZATION AND HOMOLOGOUS RECOMBINATION, *Brian R. Stanton and Luis F. Parada*, Molecular Embryology Group, NCI-Frederick Cancer Research Facility, Frederick, MD, 21701

Expression of the *Nmyc* gene has been implicated in the regulation of murine growth and development. We have analyzed the sites of *Nmyc* expression during development using *in situ* hybridization and shown that this gene is expressed at the highest levels in the developing nervous system. Comparative studies of *Nmyc* and c-*myc* show a mutually exclusive pattern of expression for these two proto-oncogenes. We have used homologous recombination in Embryonic Stem (ES) cells to introduce null mutations into one allele of the *Nmyc* locus using a gene fusion approach for selecting recombinant cell lines. Use of these cell lines will allow us to gain a better understanding of *Nmyc* regulation and function in embryogenesis. We are currently introducing these mutated ES cell lines into host blastocysts with the aim of generating germ line chimeras and mouse lines bearing a null *Nmyc* allele. We are also ablating the second *Nmyc* allele *in vitro* in order to study the effect of complete loss of *Nmyc* expression in chimeric animals. Our progress in the study of chimeric animals reconstituted with $Nmyc^{+/-}$ and $Nmyc^{-/-}$ ES cells will be presented.
Research sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with BRI.

D 219 A HAEMOPHILUS-BASED TRANSFORMATION SYSTEM FOR THE EFFICIENT RECOVERY OF MARKERS FROM MAMMALIAN SOMATIC AND GERMLINE TISSUES

Catherine Williams and Steven Weaver, Dept. of Biological Sciences, Laboratory for Molecular Biology, University of Illinois, Chicago, IL 60680

We have developed a new approach to retrieve segments of the mammalian genome from somatic and germline tissues with high efficiency. The method allows the recovery of segments that are rare and that lack an easily selectable phenotype in the mouse. There are two components to this strategy: transgenic mice carrying selectable markers from Haemophilus and the Haemophilus transformation system. Haemophilus species have the ability to take up selectively and integrate homologous DNA, even in the presence of vast excesses of competing (mouse) DNA. Competent Haemophilus are transformed with unfractionated genomic DNA from mice transgenic for a drug resistance gene from Haemophilus. The Haemophilus strain will carry a resident plasmid with sequences homologous to the integrated fragment so that the marker is recovered on the plasmid. We present evidence to confirm that modification of Haemophilus sequences by passage through the mouse genome will not affect the efficiency of uptake and that quantitative recovery of the integrated fragment can be achieved. We will apply this method to the study of germline gene conversion at the level of individual gametes. We are constructing transgenic mice in which the integrated DNA acts as a substrate for gene conversion. Thus, gene conversion events occurring in the mouse germline can be detected by transformation of Haemophilus with unfractionated gametic DNA.

D 220 MINI-MOUSE AND PYGMY ARE ALLELIC BY MOLECULAR AND PHENOTYPIC ANALYSIS, X. Xiang, K. Benson, and K. Chada, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854. The foreign DNA injected into the fertilized mouse egg can integrate and disrupt a locus which may lead to a mutation. A founder transgenic mouse segregated progeny that exhibited two different integration patterns of the foreign DNA, line A and B, which were found to be at the same locus. When mice were made homozygous for the disrupted locus within each line, the homozygotes (mini-mice) were found to be of an aberrant phenotype. They weighed 40% of their hemizygous and wild-type littermates, had short-ears, slightly disjointed faces and were sub-fertile. They are proportionately reduced in overall size and in the weight of all tissues except the brain and adrenals which are larger and smaller, respectively, than expected.

The power of this method of mutagenesis is that it allows the cloning of the disrupted locus, using the foreign DNA as an initial probe. Following this procedure, the locus was cloned and mapped to chromosome 10 where a spontaneous mutant, pygmy, which had a similar phenotype as the insertional mutant, had been previously localized. By molecular and genetic studies it was shown that the insertional and spontaneous mutants were allelic. Growth hormone levels are normal in the mutants so these mice should be an invaluable model for elucidating the biochemical nature of the human pygmy and growth hormone-resistant human dwarf syndromes.

D 221 INACTIVATION OF THE c-abl PROTO-ONCOGENE IN THE MURINE GERMLINE. Victor L.J. Tybulewicz, Tanya L. Awabdy and Richard C. Mulligan, Whitehead Institute and Department of Biology, MIT, Cambridge MA 02142.

The c-abl proto-oncogene is the cellular homologue of v-abl, the transforming oncogene of Abelson murine leukemia virus. It encodes a tyrosine-specific protein kinase located primarily in the nucleus. Its normal cellular function is unknown and to elucidate this, we are trying to create cell lines and/or animals missing the c-abl gene. We constructed an insertional targetting vector carrying the neo gene within one of the tyrosine kinase domain exons, hoping to create a null allele. On electroporating the construct into ES cells we selected out clones that were G418r. A surprisingly high fraction of these (as many as 1/25) carried the desired recombination. We have made chimaeras with these ES lines and are presently breeding them to ascertain germline contribution.

Gene Therapy

D 400 SOMATIC CELL GENE REPLACEMENT THERAPY: TREATMENT OF HEMOPHILIA B, Jonathan H. Axelrod, Raphael Scharfmann and Inder M. Verma, MBVL, The Salk Institute, P.O. Box 85800, La Jolla, CA 92138.

Therapeutic long term treatment of inherited disease may one day be possible through the use of specially designed vectors to introduce corrective genes into cells of affected patients. To explore the effectiveness of retroviral vectors to provide long term therapeutic treatment we are using the model of the inherited bleeding disorder, Hemophilia B, for which there is an animal model in dogs. The main venue for gene transfer being tested here is that of primary skin fibroblasts which after transduction are imbedded in a matrix of collagen and implanted subcutaneously in the subject. Recombinant viruses have been used to introduce a functional canine factor IX cDNA gene to skin fibroblasts from nude mice and hemophilic dogs. The transduced cells produce high levels of canine factor IX *in vitro* (0.5 to 0.8 $\mu g/10^6$ cells/24 hours), approximately 90 percent of which is enzymatically active. Experiments in hemophilic dogs are in progress and results will be presented.

D 401 FACTORS AFFECTING RETROVIRUS MEDIATED GENE TRANSFER TO MURINE AND PRIMATE PLURIPOTENT HEMATOPOIETIC STEM CELLS. David M. Bodine, Kevin T. McDonagh, and Arthur W. Nienhuis, Clinical Hematology Branch, NHLBI NIH Bethesda MD 20892

Efficient retrovirus mediated gene transfer to hematopoietic cells requires that the target cell be in the cell cycle, and a high concentration of infectious particles. We have attempted to increase the efficiency of gene transfer by inducing stem cell cycling using hematopoietic growth factors. The combination of IL-3 and IL-6 increases murine primitive progenitor cell (CFU-S) cycling and stem cell repopulating ability. The combination of IL-3 and IL-6 increased retrovirus mediated gene transfer to CFU-S and bone marrow stem cells 3 and 5 fold over that seen with IL-3 alone. The average copy number of recombinant proviruses in bone marrow stem cells of 31 positive animals was ~ 0.1 copies per genome. Retroviral titer can be increased by superinfection of a producer cell line with recombinant retrovirus (Bestwick et al. PNAS 85, 5404; Danos and Mulligan PNAS 85, 6460). We used this strategy to amplify the titer of a cell line producing the N2 retrovirus from 10^6 infectious particles/ml to greater than 10^{10} infectious particles/ml. This cell line also produces ~ 10^4 replication competent viruses/ml. Murine or Rhesus monkey bone marrow cells were cocultivated with either the high titer cell line or the original, lower titer cell line for 6 days and used to reconstitute genetically anemic or lethally irradiated recipients respectively. Three to 4 months post transplantation, the N2 provirus was detected in 10/12 mice and 3/3 monkeys infused with marrow cocultured with the high titer producer cell line, as opposed to 2/13 mice and 0/3 monkeys infused with marrow cocultured with the lower titer producer cell line.

D 402 TRANSFECTION OF PRIMARY HUMAN KERATINOCYTES WITH FUNCTIONAL GENE CONSTRUCTS IN EBV-BASED EPISOMAL EXPRESSION VECTORS. Lars Bolund, Thomas Jensen, Andrea Ballabio and Peter K.A. Jensen, Institute of Human Genetics, University of Aarhus, DK 8000 Aarhus C, Denmark.

Primary human keratinocytes from epidermal explants can be made to form a well differentiated multi-layered tissue in culture. By incubating the tissue culture in low calcium medium the differentiated cell layers can be stripped off leaving a basal cell monolayer, which is suitable for transfection experiments. The basal cells can be transfected with a surprisingly high efficiency (more than 1%), and the expression of marker genes is high when introduced in an EBV-based episomal vector with SV 40 promoters. We are presently trying to expand the transfected cell population during tissue regeneration with a combination of growth stimulation and hygromycin selection. The long-term goal is to study the genetic control of keratinocyte differentiation and to develop a model system for epithelial protein production and somatic gene therapy.

Keratinocytes from patients with X-linked ichthyosis express a pathological phenotype in tissue culture. We have inserted the cDNA for steroid sulphatase, which is deficient in X-linked ichthyosis, in the expression vector and obtained production of the enzyme. We are now trying to correct the pathological phenotype by transfecting the abnormal keratinocytes with the functional gene construct.

D 403 IN VIVO AND IN VITRO MODELS FOR ANALYSIS OF RETROVIRAL VECTOR-MEDIATED EXPRESSION OF HUMAN ADA IN ADA DEFICIENT CELLS FROM PATIENTS AFFECTED BY SEVERE COMBINED IMMUNODEFICIENCY. C.Bordignon, P.Hantzopoulos, L.Fumagalli, S.Rossini, G.Ferrari, G.E.Ungers, M.Soldati, R.Giavazzi, F.Mavilio and E.Gilboa. Istituto Scientifico H.S.Raffaele, Milano, Italy; Istituto Negri Bergamo, Bergamo, Italy; and Memorial Sloan-Kettering Cancer Center, New York, NY.

Deficiency of the enzyme adenosine deaminase (ADA) results in a variant of severe combined immunodeficiency (SCID). A new retroviral vector called double-copy (DC) vector was utilized to introduce the human ADA minigene into bone marrow cells and peripheral blood lymphocytes obtained from patients affected by ADA deficient SCID. In this vector construct the minigene of human ADA was inserted within the U3 region of the 3' LTR. Consequently, in the infected cells the minigene is duplicated and transferred to the 5' LTR (Hantzopoulos et al. PNAS 86:3519,1989). Efficiency of gene transfer and expression of this vector was compared to that of conventional vector constructs in which the ADA minigene was placed between the two viral LTRs. Freshly isolated bone marrow cells were subjected to multiple infection cycles with cell-free vector containing supernatants at high vector to cell ratio (2-5 cfu/BM cell) and than maintained in long term marrow culture (LTMC) for 4-6 weeks. On the course of the LTMC, the vector encoded h-ADA was expressed efficiently in the progeny of cultured marrow cells, reaching levels of 15-100% of the level in normal cells. Southern blot analysis revealed that efficiency of gene transfer was proportional to the number of infection cycles, and was associated with a proportional increase in the level of ADA activity in the transduced cells. Subcultures of cells fractions derived from LTMC in media selectively promoting growth of lymphoid and myeloid cells, indicated efficient expression of the h-ADA gene in both lineages. However, consistently higher expression was observed when the vector used was DCA. Efficient ADA transduction restored the capacity of subcultured lymphocytes to respond to PHA and IL-2 at levels comparable to those of normal cells. Samples obtained from these cultures were utilized to reconstitute immunodeficient mice. In the spleen of selected recipients, DCA vector-derived expression of human ADA was identified by Cellogel analysis. Furthermore, only recipients of ADA deficient human cells transduced with DCA vector showed detectable levels of human IgG in their peripheral blood.

D 404 INTRODUCTION AND EXPRESSION OF THE E.COLI β-GALACTOSIDASE GENE IN HUMAN AND MONKEY HEMATOPOIETIC CELLS, Kathy B. Burck,[1] Steve H. Bartlemez,[2] Robert G. Andrews,[2] Irv D. Bernstein,[2] A. Dusty Miller.[1] [1]Program in Molecular Medicine, [2]Division of Pediatric Oncology, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Primate hematopoietic stem cells have proved difficult to infect with retroviral vectors. Using the selectable marker neomycin phosphotransferase, it has proved possible to infect human and monkey hematopoietic progenitor cells *in vitro* with reasonable efficiency (10-30%); using a G418 preselection step reported efficiencies of infection have increased up to 3-fold. However, long-term reconstitution of adult large animals demonstrating infection of hematopoietic stem cells has not been reported, although such experiments have been successful in murine systems. We have developed a retroviral vector, LZSN, that expresses the E.coli *lacZ* gene for β galactosidase at high efficiency from a MoMuLV LTR promoter. This marker may prove more sensitive for detection of low levels of exogenous gene expression from infected bone marrow stem cells. With this vector, bone marrow progenitors expressing the *lacZ* gene after infection can be detected by a number of simple assays for β galactosidase. Results of short- and long-term expression *in vitro* of the β galactosidase gene in human and baboon hematopoietic progenitors will be presented as a prelude to long-term reconstitution of baboons with stem cells infected with the LZSN vector.

D 405 SEEDING OF INTRAVASCULAR STENTS WITH LACZ AND T-PA TRANSDUCED ENDOTHELIAL CELLS, David A. Dichek, Richard F. Neville, James A. Zwiebel, Scott M. Freeman, Martin B. Leon, and W. French Anderson, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. 20892.

Sheep endothelial cells were transduced with retroviral vectors containing either the E. coli LacZ gene or the human tissue plasminogen activator (t-PA) gene. High level expression of the inserted genes was confirmed by X-Gal staining and by an ELISA for human t-PA antigen, respectively. Transduced cells were seeded onto tubular stainless steel intracoronary stents and grown until stent coverage was complete, as assessed by phase contrast and fluorescence microscopy. Continued expression of the inserted genes by the confluent monolayers of cells on the stents was confirmed. The stents were expanded in vitro by balloon inflation and cell retention was assessed. A majority of the cells remained adherent to the stent surfaces following expansion. The coating of intravascular stents with gene-engineered endothelial cells may allow improvement of stent function through the localized delivery of pharmacologically active proteins.

D 406 EXPRESSION OF LYMPHOKINE GENES USING RETROVIRAL VECTORS, Martin A. Eglitis[1], Stanley Friedman[1], Jeanne R. McLachlin[1], Robert C. Moen[1], Daniel Kuebbing[1], J. Anthony Thompson[1], Paul Tolstoshev[1], W. French Anderson[2], and Yawen Chiang[1], [1]Genetic Therapy, Inc., Gaithersburg, MD 20878 and [2]Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892. A new vector backbone, G1, has been designed for the transfer and expression of clinically relevant genes into somatic tissues. This vector combines sequence alterations which reduce recombinogenesis with a multiple cloning site facilitating gene insertion in a consistent manner. These characteristics make this vector attractive from the perspective of safety concerns when considering retroviral-mediated gene transfer for the purpose of gene therapy. To test the behavior of this vector system, we have constructed a series of vectors containing cDNAs for either human interleukin-2 (IL-2) or human tumor necrosis factor α (TNFα). Comparisons have been made between gene expression under the regulation of the retroviral LTR and gene expression under the regulation of one of a number of internal promoters. High titer producer clones derived from the PA317 packaging line were isolated. Significant quantities of TNFα or IL-2 were detected in the supernatant of producer lines generating the respective lymphokine vector. Vector supernatants were then used to transduce target cells. Significant amounts of either TNFα or IL-2 were detected in NIH 3T3 cells following their exposure to the corresponding vector. These vectors will be significant in consideration of efforts to augment tumor infiltrating lymphocyte (TIL) function in protocols for adoptive immunotherapy of certain cancers. Expression of certain relevant lymphokines may improve TIL function in these cancer therapies, although final determination will require clinical testing *in vivo*.

D 407 IN UTERO GENE TRANSFER AND EXPRESSION OF Neo^R GENE IN MONKEYS, D. Ekhterae, N. Slotnick, R. Moen, B. Duncan, M.R. Harrison, W.F. Anderson and E.D. Zanjani, VAMC, Reno, NV, Univ. of California, San Francisco, CA, and NHLBI, Bethesda, MD We report the in vivo transfer and expression of the bacterial Neo^R gene in 2 Rhesus monkeys using the in utero retroviral-mediated gene transfer protocol without the use of cytoablative procedures. Circulating hematopoietic cells obtained from two 140 day-old fetuses (25111 & 25112) were transduced by incubation with the N2 vector (helper-free) following a period of exposure to rHuIL-3, rHuIL-6, rHuGM-CSF and PHA-LCM. The cells were washed and reinfused into each fetus. Cells were obtained from both animals at birth (cord blood) and at 1 month of age (marrow) and cultured in the presence and absence of G418 (2 mg/ml). No G418 resistant colonies were detected in cord blood samples. However, marrow cells from both newborns exhibited significant numbers of G418-resistant progenitors (controls: 0%; #25111: 0.8%; #25112: 5%). These results demonstrate the successful transfer of the Neo^R gene in monkeys using the in utero retroviral-mediated gene transfer protocol.

D 408 PSEUDOTYPE FORMATION OF RETROVIRUS VECTORS CONTAINING THE GLYCOPROTEIN OF VESICULAR STOMATITIS VIRUS, N. Emi, J.-K. Yee and T. Friedmann, Dept. of Pediatrics, Division of Molecular Genetics, Univ. Calif. San Diego, La Jolla, CA 92093. Mixed infection of a cell by Vesicular Stomatitis virus (VSV), a member of the rhabdovirus family, and retroviruses is known to result in the production of progeny virions bearing the genome of one virus encapsidated by the envelope proteins of the other virions. Such phenotypically mixed viruses have been termed pseudotypes, and they form plaques on appropriate indicator cells and can be neutralized by sera raised against the specific envelope protein. Pseudotype formation is believed to involve a specific interaction of the nucleocapsids with the envelope proteins rather than through random interaction with proteins on the cell surface to produce the envelope of budding virus particle. In the present study, we have used Moloney murine leukemia virus (MoMLV)-based retroviral vectors containing the gene for neomycin phosphotransferase to investigate the specific interaction between the VSV glycoprotein G and the nucleocapsid of retrovirus in the formation of MoMLV(VSV) pseudotypes. Pseudotyped retroviral particles were generated by co-transfection of a retroviral vector containing the gene encoding VSV G protein and a plasmid containing the MoMLV gag and pol genes into baby hamster kidney (BHK) cells. Transiently produced viruses could give rise to G418-resistant colonies in BHK cells that bear no retrovirus receptor and could not be infected by amphotropic retroviruses. Our results indicate that VSV G protein alone in the absence of other VSV-encoded proteins is sufficient to interact with the nucleocapsid of MoMLV in the formation of MoMLV(VSV) pseudotypes, and G proteins can be incorporated into the virions of retrovirus as efficiently as MoMLV envelope proteins.

D 409 SYSTEMIC DISTRIBUTION OF APOLIPOPROTEIN E SECRETED BY GRAFTS OF EPIDERMAL KERATINOCYTES, Elizabeth S. Fenjves, David A. Gordon, David L. Williams and Lorne B. Taichman, Departments of Oral Biology and Pathology and Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794.

There is considerable interest in the use of epidermal keratinocytes as potential targets for somatic gene therapy. This approach, depends on whether an epidermally synthesized protein can achieve systemic distribution. Epidermal keratinocytes in culture synthesize and secrete apolipoprotein E (apoE). In the present study, the fate of human apoE was monitored in athymic mice bearing human epidermal grafts. Human apo E was observed in the systemic circulation of graft-bearing animals as long as the graft remained on the animal (8 weeks), and was not detectable within 24 hr of graft removal. This indicates that proteins as large as apoE (299 amino acids) can traverse the epidermal-dermal barrier and reach the circulation. These results reinforce the feasibility of using genetically-altered keratinocytes grafts for the delivery of secreted proteins.

D 410 GRAFTING FIBROBLASTS GENETICALLY MODIFIED TO PRODUCE L-DOPA IN AN ANIMAL MODEL OF PARKINSON'S DISEASE, L.J. Fisher, H.A. Jinnah, M.B. Rosenberg, P.J. Langlais, F.H. Gage, and T. Friedmann, Depts. Neurosciences and Pediatrics, Univ. Calif., San Diego, La Jolla, CA 92093. In Parkinson's disease, the degeneration of dopamine neurons in the nigrostriatal pathway leads to severe deficits in motor control. One of the most effective treatments for this disease involves the replenishment of lost dopamine stores by systemic administration of the precursor to dopamine, L-DOPA. An animal model of Parkinson's disease can be produced by lesioning nigrostriatal neurons on one side of the brain with the neurotoxin 6-hydoxydopamine. The result is a unilateral motoric impairment which causes the animal to run in a circular pattern in response to drugs which stimulate locomotor activity, such as apomorphine or amphetamine. The degree of impairment can then be assessed by quantifying the number of rotations made. Our approach to reducing rotational abnormalities in these animals involves the genetic modification of fibroblasts to produce L-DOPA by introduction of the gene for tyrosine hydroxylase. This enzyme, in the presence of the cofactor tetrahydrobiopterin, catalyzes the conversion of tyrosine to L-DOPA. Fibroblasts of the 208F and Rat1 cell lines, and primary rat fibroblasts were infected with a retrovirus vector containing the rat tyrosine hydroxylase cDNA and the Tn5 neomycin-resistance gene. G418-resistant colonies were established, and the clone expressing the highest TH enzymic acitivity was selected for further study. When supplemented with pterin cofactors in vitro, cells infected with the TH vector produced L-DOPA and released it into the culture medium. In addition, when grafted to the brains of rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway, infected cells reduced drug-induced rotation. Uninfected control fibroblasts did not produce L-DOPA and had no effect on the rotational responses of lesioned animals. These results suggest the utility of an approach which combines gene transfer techniques with intracerebral grafting for the treatment of some neurological diseases

D 411 DEXAMETHASONE REGULATED EXPRESSION OF MOUSE VL30 RETROTRANSPOSONS IN RAT CELLS UPON INFECTION WITH RECOMBINANT MoMLV VIRUSES, M. Hatzoglou, C.P. Hodgson, F. Mularo, F. Bosch and R.W. Hanson, Department of Biochemistry and Pew Center for Molecular Nutrition, Case Western Reserve University, Cleveland, OH 44106 and Edison Animal Biotechnology Center and Department of Dairy Science Ohio State University, Wooster, OH 44691

Mouse retroelement (VL30) sequences were efficiently co-transferred from mouse Ψ2 cells (NIH 3T3-based packaging line) into FTO-2B rat hepatoma cells by murine leukemia virus (MoMLV)-derived retroviral vectors. The VL30 provirus was integrated into the rat genome, expressed at high levels and its transcription induced 40--fold by dexamethasone. The high efficiency of transfer and expression of the VL30 gene permitted the detection of VL30 RNA in hepatoma cells in the absence of selection from the expression of the co-transferred amino-3'-glycosyl phosphotransfease (neo) gene. Co-packaging of the VL30 retroelement occurred primarily in Ψ2 cells containing MoMLV retroviral vectors longer than 5 kb. Additionally, the regenerating liver from an adult rat, which was infected with a defective MoMLV-derived retrovirus, expressed VL30 RNA. The results demonstrate that VL30 contamination can be a significant problem with retroviral vector systems using NIH 3T3 cells as packaging cell lines. In addition, VL30 may be a useful vector system for transfer of foreign genes into mammalian cells since it has a high efficiency of transfer and its transcription can be markedly stimulated by glucocorticoids.

D 412 PHARMACOLOGICALLY INDUCED BEHAVIORAL PHENOTYPE IN HPRT - DEFICIENT MICE.
H.A. Jinnah, F.H. Gage, and T. Friedmann, Depts. Neurosciences and Pediatrics, Univ. Calif., San Diego, La Jolla, CA 92093. Congenital HPRT deficiency in humans results in a severe neurological disorder known as the Lesch-Nyhan syndrome. Recently, two research groups independently produced HPRT-deficient mice as animal models for this disorder, but surprisingly, these mice do not spontaneously exhibit any abnormal behaviors. Unfortunately, the apparent absence of an abnormal behavioral phenotype in these mice has raised questions concerning their utility as relevant neurological models of the Lesch-Nyhan Syndrome. Although HPRT-deficient mice do not exhibit abnormal behaviors spontaneously, they might have "subclinical" abnormalities which could be revealed by appropriate pharmacological agents. In the present study, we tested the sensitivity of HPRT+ and HPRT- mice to amphetamine, a drug which stimulates the release of catecholamine neurotransmitters and can cause Lesch-Nyhan-like self-injurious behavior in normal rodents. Initially, mice (n=20) were scored for the occurrence of several behaviors using a behavioral rating scale. After subcutaneous injection of 8 mg/kg amphetamine, the predominant response of HPRT+ animals was pronounced locomotor hyperactivity. HPRT- animals, however, exhibited transient hyperactivity followed by a phase of persistent stereotypic behaviors such as gnawing, sniffing, and licking, with little or no locomotor activity. In order to obtain a more objective and quantitative measure of this behavior, a second group of mice (n=20) was tested for amphetamine-stimulated motor activity in photocell chambers. After a dose of 8 mg/kg, the activity profiles of HPRT+ and HPRT- animals were strikingly different. HPRT+ animals displayed a single peak of activity which subsided after 2 hours, while HPRT- animals exhibited biphasic activity profiles, indicative of the intervening phase of stereotypic behavior observed in the previous experiment. These results demonstrate the existence of a reproducible abnormal behavioral phenotype in HPRT-deficient mice, and suggest the existence of an underlying abnormality of catecholamine systems in the brains of these animals.

D 413 INTRON-DEPENDENT EXPRESSION OF THE HUMAN PURINE NUCLEOSIDE PHOSPHORYLASE GENE.
Jon J. Jonsson, Nancy W. Shilling, R. Scott McIvor. Institute of Human Genetics, Department of Laboratory Medicine and Pathology. University of Minnesota, Minneapolis, MN 55455.

Purine nucleoside phosphorylase (PNP) catalyzes the formation of nucleobase and pentose-1-phosphate from inosine and guanosine nucleosides, and is an important enzyme in the catabolism and recycling of purine bases. Absence of PNP activity in humans, which is inherited in an autosomal recessive manner, is associated with a severe T-cell immunodeficiency and is considered a prototype disease for gene therapy. The human PNP gene consists of six exons dispersed across approximately 10 kb of DNA. The moderate size of the human PNP gene makes it convenient for investigating the role of natural regulatory elements in optimizing gene expression.

We previously characterized the PNP promoter and found that different sequence lengths extending up to 2,243 bp from the 5' untranslated region of the human PNP fused to the bacterial chloramphenicol acetyltransferase gene gave only a two fold variation in levels of transient expression after transfection into NIH 3T3 fibroblasts.

To determine the role of introns in human PNP gene expression, a series of plasmids were constructed to contain the 2.2 kb PNP promoter and the PNP coding sequence along with a variable number of PNP introns. Transient expression in NIH 3T3 fibroblasts was assayed by isoelectric focusing of cell lysates to separate human from endogenous murine PNP followed by histochemical staining for PNP activity. Human PNP activity was not detectable in cells transfected with a construct containing the cDNA sequence alone (no introns). Constructs containing intron #1 or introns #1 and #2 resulted in a substantial human PNP activity (1x). This level of expression was also observed in a construct containing only 0.7 kb of intron #1 (normal size 2.9 kb). The genomic construct containing all five introns gave the highest activity (1-2x). Interestingly constructs containing introns #3 to #5 or introns #1, #4, and #5 resulted only in a minimally detectable PNP signal (0.2x), suggesting a role for correct order of intron splicing for optimal expression. The PNP intron #1 enhancement of expression was also seen using a heterologous promoter, the Rous sarcoma virus long terminal repeat, instead of the PNP promoter. The mechanism of intron-dependent PNP gene expression is currently under investigation.

D 414 HEMATOPOIETIC GROWTH FACTORS INCREASE RETROVIRAL VECTOR-MEDIATED GENE TRANSFER INTO HUMAN PROGENITOR CELLS, Kohn D.B. and Nolta J.A., Childrens Hospital of L.A, USC School of Medicine, Los Angeles, CA. The hematopoietic growth factors (HGF), IL-3, IL-6, and GM-CSF, have been shown to stimulate proliferation of multi-lineage primitive hematopoietic progenitor cells (HPC). We therefore have compared the ability of HGF to increase retroviral vector (RV)-mediated gene transfer rates into human HPC. Marrow was pre-cultured for 48 hours with HGF, singly or in combinations, and then infected with the N2 RV. Gene transfer rates were determined by quantitation of G418-resistant CFU. Gene transfer was enhanced by 48-hour pre-culture in either GM-CSF or IL-3 alone (but not IL-6) and by each combination of HGF. IL-3 plus IL-6 consistently produced significantly higher rates of G418-R CFU (55-65% vs 25% unstimulated) than any other combination of HGF tested. The ability of the different HGF to increase RV-mediated gene transfer correlated closely with the induced increase in HPC cycling, measured by hydroxyurea (HU) progenitor inhibition. Addition of IL-3 plus IL-6 to bone marrow enabled a two-fold increase in the total number of CFU formed (maximal by 30 hours after adding HGF), followed by peak sensitivity of HPC to HU inhibition and RV infection (at 50 hours). These results suggest that IL-3 plus IL-6 cause resting HPC to synchronously leave G_0 and begin active cell cycling, with maximal RV infection occurring during S-phase. Marrow, pre-stimulated with HGF prior to RV infection, was then maintained in LTBMC for up to 2 months. The levels of G418-R HPC remained stable and no apparent depletion of stem cell content resulted from the prior exposure to highly stimulatory doses of HGF. The ability to achieve high rates of RV mediated gene transduction of long-lived human HPC, by pre-stimulation with appropriate combinations of HGF, will be important for gene therapy of genetic diseases.

D 415 POSITIVE SELECTION OF HUMAN HEMATOPOIETIC STEM CELLS USING THE AIS STEM STEM CELLECTOR. Jane S. Lebkowski, Lisa Schain, Vibeke Strand, David Warren+, Roland Levinsky*, and Thomas Okarma. Applied ImmuneSciences, Inc. Menlo Park, CA; +Memorial Sloan Kettering Cancer Center, New York, NY; *The Institute for Child Health, London, UK.

We have developed a simple procedure to positively select for human hematopoietic stem cells using a well characterized CD34 monoclonal antibody and the AIS Stem CELLector device. The Stem CELLector device is a sterile polystyrene flask which contains the CD34 monoclonal antibody covalently bound to the internal surface. In this procedure, unfractionated human bone marrow nucleated cells are incubated in the device for one hour at room temperature. After incubation, the adherent stem cells are recovered by a simple physical process and are available for further use. The enriched CD34+ stem cells are greater than 85-90% viable and retain their ability to proliferate and differentiate. As measured by a variety of in vitro colony forming assays, the purified CD34+ cells are 10-50 fold enriched in both myeloid and erythroid progenitors. The long term hematopoietic activity of these cells is currently being defined in preparation for their use in bone marrow transplantation.

D 416 DEVELOPMENT OF GENE THERAPY FOR THE TREATMENT OF AIDS, James Mason*, Richard A. Morgan*, David J. Looney **, Daryl D. Muenchau*, Flossie Wong-Staal**, Robert C. Gallo**, and W. French Anderson*. *NHLBI, **NCI, National Institutes of Health, Bethesda, MD 20892.

Retroviral vectors have been developed which produce a secreted form of the helper/inducer T cell antigen, CD4. Amphotropically packaged vectors were used to transduce cells (including several transformed cell lines as well as primary fibroblast an endothelial cells) and these cells were shown to express the secreted CD4 (sCD4) gene product. The sCD4 produced by the viral vectors is immunoprecipitated by monoclonal antibodies against CD4 which specifically block HIV infection of helper/inducer T-cells. direct physical interaction of vector-produced sCD4 and HIV-1 gp120 was demonstrated by coprecipitation of sCD4/gp120 with antiserum directed against HIV gp120. Further, transduced cells producing sCD4 can protect HIV susceptible cells from infection by HIV. We have demonstrated that retroviral vectors can be constructed which express sCD4 and tha cells transduced by these vectors can protect cells from HIV infection in culture. sCD4 retroviral vectors could potentially be used to engineer the cells of an AIDS afflicted individual, and thus these data are a model for a potential gene therapy approach for the treatment of AIDS.

D 417 A METHOTREXATE-RESISTANT DIHYDROFOLATE REDUCTASE OBTAINED FROM HIGHLY DRUG-RESISTANT MOUSE L51878Y CELLS; cDNA ISOLATION AND SCREENING BY TRANSFECTION IN CULTURED MAMMALIAN CELLS, R. Scott McIvor* and Christian C. Simonsen**, *Institute of Human Genetics and Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis MN 55455, and **Department of Molecular Biology, InVitron Corporation, Redwood City, CA 94067. Methotrexate is a potent folate antagonist and a useful cancer chemotherapeutic agent. Resistance to MTX in mammalian cells can occur as a result of the production of increased or altered (MTX-resistant) DHFR activity. To isolate the cDNA sequence encoding a MTXr-DHFR expressed in murine L5178Y cells adapted to grow in 1 mM MTX (kindly provided by J. Goldie), RNA was extracted and cDNA synthesized using a specific primer complementary to a sequence in the 3' untranslated region of the murine DHFR message. The DHFR coding sequence was then amplified using the polymerase chain reaction and a sense primer from the 5' untranslated region. cDNA thus generated was directionally inserted into a mammalian expression plasmid at a site between the mouse metallothionein promoter and a polyadenylation signal derived from the hepatitis B virus surface antigen gene. DHFR inserts (approx. 1/3 of all bacterial colonies) were verified by restriction analysis. Several of these plasmids were transfected into mouse 3T3 tk^- (lacking thymidine kinase) cells and mammalian cell clones resistant to 0.1 μM MTX isolated. The DHFR activity contained in extracts from one of these clones exhibited MTX inhibition kinetics which were displaced 50-fold higher in comparison with extracts from normal mouse 3T3 cells, indicating the presence of a MTXr-DHFR sequence on the newly introduced plasmid. This drug-resistance gene may prove to be useful in gene transfer studies in animals and in cultured mammalian cells.

D 418 ENRICHMENT OF MURINE HEMATOPIETIC STEM CELLS: RECONSTITUTION AFTER SHORT TERM CULTURE. Maureen A. McNally, Jane S. Lebkowski, Sheila Finch Manzagol, S.Anne Pletcher, Lisa R. Schain, and Thomas B. Okarma. Applied ImmuneSciences, Inc. Menlo Park, CA.

A method is described to purify murine hematopoietic stem cells. The procedure involves fluorescence activated cell sorting of nonadherent nucleated bone marrow cells for the presence of the antigen, Thy1.2, and the absence of the lineage specific antigens, Lyt2, L3T4, Mac1, B220, and J11D.2. These Thy1.2+T-B-M-J- cells are 200-1000 fold enriched in both in vitro soft agar colonies and spleen colony forming units. Moreover, this marrow subset shows enhanced ability to reconstitute lethally irradiated mice. These purified pluripotent stem cell populations also have enhanced potential for the rescue of lethally irradiated haplotype mismatched mice. Experiments concerning the short and long term culture of these purified stem cells will be presented.

D 419 DEVELOPMENT OF HERPES SIMPLEX VIRUS VECTORS FOR GENE TRANSFER, A. Miyanohara, P.A. Johnson, R.Ramos, K.Yoshida, F.H. Gage and T. Friedmann. Depts. Pediatrics and Neurosciences, UCSD, La Jolla, CA 92093. We have been studying the feasibility of using mutants of HSV-I as vectors for gene transfer. The initial mutant we used, D30EBA (Paterson and Everett, Glasgow University), has a 3.6 kb deletion in the coding sequence of immediate-early gene 3 (IE 3), which is essential for virus growth and can be propagated in cells which supply the IE 3 gene product in trans. The E.coli lacZ gene, driven by the HCMV IE control region, was inserted into an intergenic site of the D30EBA genome to create the vector 'Cgal'. We have found efficient infection and good expression of lacZ in a variety of cell types including primary cultures of hepatocytes, fibroblasts and neuronal cells. The ability of the HSV vector to infect cell types with high efficiency that are refractory to CaPO4-transfection or retrovirus-mediated gene transfer demonstrates the potential usefulness of this virus for gene transfer to cells derived from such organs as the brain and the liver. Therefore, we replaced the lacZ gene in Cgal with cDNA encoding the human LDL receptor (h-LDL-R), a deficiency in which is responsible for familial hypercholesterolemia. This virus was used to infect human hepatoma and fibroblast cell lines. Strong expression of h-LDL-R RNA was detected by Northern analysis in both cell lines.

However, in all cell types studied cytopathic effect (CPE) was observed after 1-3 days infection with D30EBA-derived vectors, despite the abence of replication-competent virus. Preliminary data suggested that this may be due to continued HSV gene expression in the absence of IE 3. We are currently examining different mutant viruses for reduced toxicity.

D 420 TRANSFER AND RECOVERY OF GENE ENGINEERED CELLS FROM HUMAN BEINGS, Richard A. Morgan*, Kenneth Cornetta*, Paul Aebersold**, Attan Kasid**, Robert Moen#, R. Michael Blaese**, Steven A. Rosenberg**, and W. French Anderson*. *National Heart, Lung, and Blood Institute, and **National cancer Institute, National Institutes of Health, Bethesda, MD 20892, #Genetic Therapy Inc., Gaithersburg, MD 20878.

The first federally-approved experiment designed to transfer gene-engineered human cells to human beings is currently in progress. In this experiment, human tumor infiltrating lymphocytes (TIL) have been genetically marked by transduction with a retroviral vector carrying the bacterial neomycin phosphotransferase gene (NeoR). These genetically marked TIl cells are then administered to the patient undergoing TIL cell cancer immunotherapy. The aim of this protocol is to track the genetically marked TIL cells once they are returned to the patient, with the hope of gaining insight into the mechanism of TIL cell mediated tumor regression. The novelty of this clinical protocol called for the development of several new tests to ascertain the safety of the procedure along with very sensitivity methods for the detection of small numbers of marked cells within the patients. Extremely sensitive biological and physical tests were developed to eliminate the possibility of contamination of retroviral vector preparations with replication competent virus. Specific polymerase chain reaction (PCR) procedures were also developed for the detection of small numbers of transduced TIL cells in the presence of large numbers of untransduced cells. We report on our analysis of blood and biopsy material from the first several patients taking part in this protocol and report that gene engineered cells can be recovered from patients weeks following transfer.

D 421 STRUCTURE/FUNCTION ANALYSIS OF MURINE RETROVIRAL ENVELOPE GENES: ASSESSMENT OF THE PROTEIN FOR TISSUE TARGETING, Daryl Muenchau, Richard Morgan, Sandra Ruscetti*, Liming Shu, James Mason, Arifa Khan**, Ofer Nussbaum, Sabine Sturm, Ann Stephens and W. French Anderson, NHLBI, NCI*, NIAID**, NIH, Bethesda, MD 20892
We have begun to study the structure to function properties of the Moloney murine leukemia virus (MMLV). Several approaches are being pursued. One analysis centers on a study of chimeric envelope genes containing defined regions of the AKV ecotropic MLV substituted for corresponding MMLV regions. The chimeric viruses, AKV and MMLV are used to rescue spleen focus-forming virus (SFFV), the replication defective agent responsible for acute erythroleukemia in mice. Infection of bone marrow cells with the MMLV/SFFV complex efficiently generates erythroid bursts in vitro in the absence of exogenous erythropoeitin while the AKV/SFFV complex does not. Chimeric helper/SFFV complexes carrying a specific 302 bp region of the AKV gp70 coding sequence fail to generate erythroid bursts while chimeras with MMLV gp70 sequences in this region efficiently generate bursts. A series of MMLV envelope insertion/deletion mutants have been analyzed for biological properties in order to identify regions necessary for transport and processing. An additional study is underway to determine the envelope region(s) responsible for receptor binding and host range properties using chimeric MMLV/4070A amphotropic envelopes and chimeric MCF/Xenotropic envelope genes. Results from these studies will be discussed with regard to the behavior of different envelope constructs and the suitability of murine retroviral envelope proteins for tissue targeting.

D 422 CONSTRUCTION OF RECOMBINANT ADENO-ASSOCIATED VIRUS THAT HARBORS HUMAN BETA-GLOBIN cDNA, Seigo Ohi, Mrinalini Dixit, M. Kelly Tillery and Steven G. Plonk, Dept. of Biochemistry and The Comprehensive Sickle Cell Center, Meharry Medical College, Nashville, TN 37208. The goal of this laboratory is to develop recombinant human parvoviruses containing normal alpha- or beta-globin cDNA/gene for gene therapy of hemoglobinopathies. Towards this goal, the human beta-globin (HbetaG) cDNA was excised out from JW102 (J.T. Wilson et al. Nucl. Acids Res. 5: 563, 1978) using S1 nuclease and ligated with AAV2 DNA in a plasmid (pAV2: C.A. Laughlin et al., Gene 23: 65, 1983) downstream of the P40 promoter. Cloning of this construct in DH5 cells resulted in isolation of a recombinant clone, pAVHbetaGHP11. Transfection via electroporation of the construct to human 293 cells resulted in expression of the beta-globin cDNA as evidenced by the synthesis of transcripts hybridizable to human beta-globin cDNA probe. Addition of the 3'-end region of AAV DNA that contains both transcription termination signal and the origin of DNA replication for AAV to the pAVHbetaGHP11 resulted in production of a clone, pAVHbetaGpsi1. Transfection of this construct to 293 cells permitted the recombinant AAV DNA containing the HbetaG cDNA to replicate inside the cell. This, in turn, resulted in production of the infectious recombinant virus. (Supported by NIH Grants HL01989 and HL38737 to SO)

D 423 GENE THERAPY: AN SV40 PSEUDOVIRAL VECTOR FOR HEMOPOIETIC CELLS, Ariella Oppenheim, Aviva Peleg, Nava Dalyot, Ziv Sandalon, Orly Shaul and Naaman Avishai, Department of Hematology, The Hebrew University - Hadassah Medical School, PO BOX 12000, Jerusalem 91200, Israel.
An SV40 pseudoviral system developed in our laboratory is very efficient in gene transfer into a variety of cells, including human hemopoietic cells. Plasmids carrying the SV40 origin of replication are encapsidated in COS (monkey kidney) cells as SV40 pseudovirions, and transmitted into the target cells by viral infection. The first group of vectors carry sequences for propagation in bacteria, and can function as shuttle vectors. These vectors are also suitable for screening promoters and enhancers in conjunction with a reporter gene, such as cat. The second group of vectors, designed for gene therapy, carry restriction endonuclease sites for the removal of the prokyaryotic sequences after the propagation in bacteria. These constructs may include as little as 200 bp of the SV40 DNA, and their cloning capacity for human DNA such as beta globin gene and flanking sequences, is over 5 kb. Thus plasmids carrying over 95% human DNA are introduced efficiently into human hemopoietic cells. A helper-free packaging cell line is presently being constructed. The efficiency of the vector in gene targetting is being assessed. The vector is most suitable for gene therapy because of its very wide host range and as high titer stocks can be readily prepared and monitored for safety and purity.

D 424 DERMAL FIBROBLAST MEDIATED GENE THERAPY FOR INHERITED DISEASE, Theo D. Palmer, Guy J. Rosman, A. Dusty Miller, Department of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 90104. It is conceivable that inherited diseases could be treated by transplanting functional genes into a patients somatic tissues thereby supplementing a missing or defective protein. In this work, a gene transfer system using helper-free retroviral vectors was used to introduce either human factor IX or human adenosine deaminase (ADA) genes into human and rat dermal fibroblasts. The engineered cells were able to produce high levels of both ADA and factor IX *in vitro* and when transplanted into host animals showed continued production of therapeutic proteins. Most animals showed easily detectable levels of protein but protein levels diminished with time. Preliminary results indicate that cells containing vector sequences may be preferentially lost when transplanted along with uninfected cells. In addition, a proportion of the cells isolated from transplanted animals contain vector sequences but fail to express protein in culture. This suggests that expression of vector sequences may be linked to cell loss in the animal. The mechanisms of this vector associated cell loss are currently being studied.

D 425 HUMAN RECOMBINANT ADENOVIRUS USED TO CORRECT A MOUSE ENZYME DEFICIENCY, Michel Perricaudet*, Jean-François Chasse°, Michèle Minet-Thurriaux°, Leslie Stratford*, Massimo Levrero*, Pascale Briand°. * Institut Gustave-Roussy Villejuif and ° LBC Necker Hospital, Paris FRANCE.
Human ornithine transcarbamylase deficiency is one of the most frequent hereditary hyperammoneamias. This X-linked inherited disease leads to the death of 75% of the affected males, whatever the treatment. Thus, although several types of mutations leading to various levels of residual activity have been described, the outcome is in most cases very dramatical. We have used the Spf-ash animals exhibiting an OTC deficiency characterized by an equal reduction in the amounts of OTC activity, OTC protein and specific OTC mRNA (but without any modification of the system leading to the mature enzyme in mitochondria), to attempt a correction of the enzymatic defect by somatic gene transfer. A human recombinant adenovirus which harbors the OTC gene under the control of the viral major late promoter has been constructed. Following the injection of the recombinant virus into the liver of mutant mice, an elevated enzymatic activity was found in several animals and this, at different times after the injection. Concomitant to the expression of the introduced OTC gene, a corrected phenotype could be observed in some animals. Experiments are underway using a recombinant adenovirus carrying the OTC gene controlled by a tissue specific promoter.

D 426 RETROVIRUS MEDIATED TRANSFER OF INDUCIBLE INTERFERON GENE WHICH SHOWS CELL SPECIFIC EXPRESSION, Paula M. Pitha, Daniel P. Bednarik and John Engelhardt
The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD 21205
In the present study, we attempted to elicit inducible tissue specific expression of an interferon gene. The targeting of cytokine gene to tumor cells, or cells that are chronically infected by viruses (HIV, HBV or EBV), could prove to be of clinical importance. To obtain selective expression in infected cells, interferon genes were placed under the direction of a viral promoter which could be trans-activated by viral infection (HIV-LTR), and the hybrid genes were transduced by amphotropic retroviruses. The lines established induced interferon after HIV or HSV-1 infection and restricted their replication. To target inducible interferon synthesis to a specific group of hematopoietic cells (B-cells), we have inserted IgEnh 5' or 3' of the human β-interferon gene, and transduced it by replication defective xenotropic virus to murine B-cells and fibroblasts cells. By deleting the enhancer in the 3' LTR, inserting the human β-interferon gene and varying placement of the IgEnh, we were able to construct vectors which yielded proviruses with different cell type specific regulation. The inducible and tissue-specific expression of β-interferon within the retroviral vector was greatly influenced by the deletion of the LTR enhancer and by the orientation of both the β-interferon gene and IgEnh within the vector. In B-cells, the insertion of the IgEnh increased constitutive expression, however, the high inducibility was achieved only in constructs containing the IgEnh 3' to the interferon gene.

D 427 GRAFTING FIBROBLASTS GENETICALLY MODIFIED TO PRODUCE NERVE GROWTH FACTOR PREVENTS NEURONAL DEGENERATION IN LESIONED RAT BRAIN, M.B. Rosenberg, M.H. Tuszynski, R.C. Hayes, K. Yoshida, D.M. Armstrong, T. Friedmann and F.H. Gage, Depts. Pediatrics and Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093. Because of its relative inaccessibility and the lack of suitable stem cell populations, the brain has received scant attention in gene therapy studies. To treat some disorders, however, it may not be necessary to introduce foreign genes into neurons themselves. Instead, it may be possible to transplant cells that provide a missing factor to neurons. In a test of this therapeutic model, we showed that rat fibroblasts, genetically modified to secrete nerve growth factor (NGF) and then grafted into rats that had received brain lesions, prevent the degeneration of cholinergic neurons two weeks after transplantation (Science 242:1575, 1988). The present studies were undertaken to evaluate longer-term graft survival and gene expression. Fibroblasts of the line Rat1 were infected with a retrovirus vector expressing mouse NGF cDNA and the Tn5 neomycin-resistance gene. Rats were given unilateral aspirative lesions of the fimbria-fornix, the fiber tract through which the axons of cholinergic neurons in the medial septum project to the hippocampus, the site of NGF synthesis. Without NGF, 50 to 60 per cent of these neurons normally would die. Infected or control fibroblasts were then grafted into the lesion cavities. Animals were sacrificed after two or eight weeks, and the brains were examined for cholinergic neuron survival in the medial septum by immunohistochemistry for choline acetyltransferase and for NGF receptors. At two weeks there was virtually complete savings of cholinergic neurons in the lesioned side of all animals that had received NGF-secreting grafts but not in animals with control grafts. At eight weeks, a majority of treated animals still exhibited cholinergic neuron survival, and in situ hybridization for NGF mRNA indicated continued transgene expression. These studies indicate that genetically modified fibroblasts can survive and express transgenes at biologically relevant levels for at least two months following intracerebral grafting. Studies are in progress to determine whether primary cultures of skin fibroblasts will also survive and function in this model.

D 428 **RIBOZYME MEDIATED INTRACELLULAR IMMUNITY TO HIV-1 IN CD4$^+$ HeLa CELLS, John J. Rossi, Edouard Cantin, Paula Ladne, Delilah Stephens, John A. Zaia, and Pairoj Chang, Department of Molecular Genetics and Divisions of Neurology and Pediatrics, Beckman Research Institute of the City of Hope and City of Hope Medical Center, Duarte, Calif. 91010** Anti-sense, catalytic RNAs or "ribozymes" represent a potentially powerful tool for gene therapy. We have exploited a type of ribozyme called a "hammerhead" to develop molecules which specifically cleave HIV-1 RNAs. One of these ribozymes has been transfected into HeLa CD4$^+$ cells using an expression vector which placed the ribozyme under the transcriptional control of the human ß-actin promoter. Transfectants expressing the ribozyme have been obtained and examined for sensitivity to HIV-1 infection. Our most sensitive assay, a PCR across the purported cleavage site, demonstrated near complete destruction of HIV-1 RNA in cells expressing this ribozyme. The PCR results, in conjunction with other biological data, strongly implicate that the intracellular ribozyme effectively protects these cells from HIV-1 infection and/or propagation. These results have important implications for the use of ribozymes in gene therapy.

D 429 THE FUNCTION OF PRIMITIVE VERTEBRATE GENES IN A MAMMALIAN GENOME. Raul A. Saavedra, Carol Readhead, Lance Fors and Leroy Hood, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125

Myelin of the vertebrate nervous system is the structure that provides electric insulation and thus facilitates the rapid conduction of nerve impulses along axons. The major myelin proteins in the mammalian central nervous system (CNS) are proteolipid protein (PLP) and myelin basic protein (MBP); whereas, in peripheral nervous system (PNS) they are protein zero (P_0) and MBP. Sharks are the most primitive living vertebrates that express a mammalian-like myelin. Sharks, diverged from other vertebrates about 400 million years ago, thus they are an appropriate system to study the evolution of the myelin genes and proteins. We extracted and sequenced the major CNS myelin proteins from the shark Heterodontus francisci. The sequence data shows that the two major proteins of shark CNS myelin are similar to mammalian P_0 and MBP. We cloned and sequenced cDNAs that code for shark P_0 and MBP. The translated sequences of these shark proteins are about 45% similar to their mammalian counterparts. The predicted secondary structures of the shark and mammalian proteins, however, show striking similarities. This conservation in structure led us to test whether the shark proteins could function in mammals. Shiverer mice bear a deletion in the MBP gene, cannot make MBP, and do not condense their myelin. DNA constructs bearing the mammalian MBP promoter, shark MBP cDNA, and mammalian splicing and polyadenylation signals were made. These plasmids are being used to make transgenic mice to study the in vivo effects of shark MBP on the shiverer phenotype. The results of these experiments will be presented.

D 430 EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN MICE AND RHESUS MONKEYS FOLLOWING TRANSPLANTATION OF BONE MARROW CELLS INFECTED WITH RECOMBINANT RETROVIRUSES, Dinko Valerio and Victor W. van Beusechem, Radiobiological Institute TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands.

Amphotropic recombinant retroviruses were generated in which sequences encoding human adenosine deaminase (hADA) are transcribed from a hybrid long terminal repeat in which the enhancer from the Moloney murine leukemia virus was replaced by an enhancer from the F101 host range mutant of polyoma virus. Hemopoietic stem cells, present in murine bone marrow cells, were infected with this virus and subsequently subjected to a selection procedure to enrich for cells expressing hADA. Following transplantation of these cells into lethally irradiated mice hADA expression was observed in the stem cell (CFU-S) derived spleen colonies. A clear relationship existed between the stringency of the selection procedure and the percentage of hADA expressing spleen colonies. Without preselection the infection resulted in approximately 10% hADA positive spleen colonies. When an optimal selection procedure was employed 7.6% of the CFU-S survived, from which 80% continued to express hADA in transplanted mice. Mice surviving such transplantation experiments expressed hADA in their blood cells over extended periods of time (8 weeks or more). To extend these studies into a model more relevant for the clinical situation we also performed autologous transplantation in rhesus monkeys using bone marrow that was co-cultured with the hADA-virus producing cells. Preliminary results indicate that the transplanted monkeys express hADA in their blood cells. This expression was observed at least until 6 weeks post-transplantation. Studies concerning the persistence and stability of these expression patterns are currently in progress.

Michael N. Gould, Department of Human Oncology and Environmental Toxicology Program, University of Wisconsin, Madison, WI 53792

The mammary gland is a potentially important target organ for somatic gene transfer. Sense and antisense constructs may be used to alter milk composition. Useful proteins can also be synthesized and released into milk or blood. In addition, the role of various normal and mutated genes in breast cancer etiology and treatment can be investigated. We have developed methodology for the introduction of genes into rat mammary epithelial cells in situ with good efficiency. High titers ($>10^8$ CFU/ml) of a replication-defective retrovirus vector, JR-GAL, containing the gene for β-galactosidase (β-gal) under control of a viral LTR, were injected into the central mammary duct of 60 day old female Wistar Furth rats. Recipients were pretreated with perphenazine, resulting in a ~10 fold increase in mammary cell proliferation. This increase in proliferation also resulted in a ~10 fold increase in functional vector integration. A single exposure of the in situ mammary cells to vector packaged in the ecotropic cell line psi-2 resulted in an integration frequency of $5x10^{-4}$. This frequency could be increased to $3x10^{-3}$ (i.e. 0.3% of all mammary epithelial cells have a functional β-gal gene) by using the amphotrophic packaging line PA-317. This frequency could potentially be increased by multiple exposures. Finally, the stability of functional integration has been investigated. The frequency of in situ mammary cells expressing β-gal one week after vector exposure was $6.5x10^{-4}$, at one month after exposure the frequency was $4.5x10^{-4}$ and at three months it was $3.3x10^{-4}$. We are currently using this vector system to introduce both oncogenes and genes for lactogenic and growth hormones into mammary cells in situ. **(Supported by CA44387.)**

D 432 RETROVIRAL-MEDIATED GENE TRANSFER TO MURINE HEPATOCYTES: VECTORS WITH INCORPORATED LIVER-SPECIFIC ENHANCER AND PROMOTER ELEMENTS, Dwayne A. Wolf and John Papaconstantinou, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550

The mouse alpha-fetoprotein (AFP) gene is normally expressed only in fetal liver and to a lesser extent in yolk sac and embryonic gut, while the albumin gene is expressed in fetal and adult liver. The liver specificity of the albumin gene is conferred largely by its promoter region. The AFP gene contains a liver-specific promoter and three distal enhancers contained within the first 7 Kb upstream of the gene. A series of retroviral vectors was constructed in which transcription of an internal reporter gene, lacZ, is directed by the mouse albumin promoter. In addition, the viral enhancer sequences in the U3 region of the downstream LTR were replaced with various fragments of AFP enhancer sequences. These sequences are duplicated to the upstream LTR during reverse transcription in the target cells. Internal sequences that confer G418-resistance allowed selection of stable producer cells and enabled the virus to be titered by correlating the amount of viral RNA in the media to the number of G418-resistant colonies produced by virus with wild-type LTR's. The packaging cell line was psi-CRE and the target cells are cultured mouse hepatoma cells (BWTG3) and NIH-3T3 fibroblasts. We are presently testing to see if beta-galactosidase production will be different in hepatoma cells relative to the fibroblasts.

D 433 RETROVIRAL VECTOR-MEDIATED TRANSFER OF β-GLUCURONIDASE INTO MUCOPOLYSACCARIDOSIS TYPE VII CELLS. J.H. Wolfe, B.F. Smith, D.G. Markowitz, G.D. Aguirre, M.E. Haskins & D.F. Patterson, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104.

Retroviral vectors which express high levels of β-glucuronidase (β-gus) activity in β-gus-deficient mucopolysaccharidosis type VII (MPS VII) (Sly disease) fibroblast cell lines were used to compare infectivity and expression in primary cells from various tissues. High-titer vector viruses efficiently infected primary cultures of canine retinal pigment epithelial (RPE) cells and canine myoblasts, which differentiated into mature myotubes. Canine MPS VII bone marrow cells that were infected at the same multiplicity of infection also expressed high levels of β-gus. However, examination of β-gus activity in individual bone marrow cells by histochemical staining indicated that only a fraction of the cells exposed to vector virus were expressing β-gus activity, even among cells that were actively dividing. By directly comparing primary cells from different tissues, these data suggest that a substantial fraction of bone marrow cells may be specifically refractory to retroviral vector infection.

D 434 DIRECT GENE TRANSFER INTO MOUSE MUSCLE IN VIVO, Jon A. Wolff, Robert W. Malone, Phillip Williams, Wang Chong, Gyula Acsadi, Agnes Jani, Philip L. Felgner, Departments of Pediatrics and Genetics, Waisman Center, University of Wisconsin, Madison, WI 53706.
The direct introduction of genetic information into muscle cells *in vivo* would be useful for gene therapy purposes. RNA and DNA expression vectors consisting of genes for chloramphenicol acetyltransferase (pRSVCAT) and luciferase (pRSVL) were separately injected into mouse skeletal muscle *in vivo*. Protein expression was readily detected in all cases; no special delivery system was required for these effects. The levels of expression from both the RNA and DNA constructs were comparable to levels of expression obtained from fibroblasts transfected *in vitro* under optimal conditions. *In situ* cytochemical staining for β-galactosidase activity was localized to muscle cells following injection of a β- galactosidase DNA vector (pRSVLac-Z). Expression from the DNA expression vectors were stable for at least two weeks and further studies are in progress to determine their long-term stability.

Gene Mapping

D 500 COMPARISON OF A RADIATION AND LONG RANGE RESTRICTION MAP SURROUNDING THE MOUSE AGOUTI LOCUS, G.S. Barsh, G.P. MacDonald, D.R.Cox, and C.J. Epstein, Departments of Pediatrics and Psychiatry, UCSF, San Francisco, CA 94143. We have undertaken a genetic and physical analysis of the 5-10 cM region surrounding the mouse agouti locus (*A*) as a first step towards understanding the molecular basis of the recessive lethal mutations a^{l}, a^{x}, a^{16H}, and A^{y}, which interfere with normal peri-implantation development. The *Src*, *Psp*, *Pyg-b*, and *Hck* genes, and insertion sites for the *Emv-13*, *Emv-15*, and *Xmv-10* proviruses are located within this region, and DNA probes for these loci have been used to detect radiation-induced agouti mutations and to generate a physical map by pulsed field gel electrophoresis and radiation hybrid mapping. We have previously reported that a 75 kilobase deletion associated with the a^{l} allele is found between *Psp* and a CpG island 400 kb away. Long range restriction maps have now been constructed that encompass more than 3000 kb and link *Emv-13*, *Psp*, and the a^{l} deletion in one segment, and *Xmv-10*, *Pyg-b*, *Emv-15*, and *Src* in a second segment. This map allows the orientation of all six markers, shows that the a^{l} deletion is located telomeric to *Psp*, and identifies a region where recombination is relatively suppressed between *A* and *Emv-15*. In order to estimate the physical distance between the two segments and to recover additional DNA markers from this region, a panel of radiation hybrid cell lines has been generated starting with a mouse/hamster hybrid specific for mouse chromosome 2. The frequencies of discordance for two-point comparisons are 5.8% (*Psp—Emv-15*), 10.3% (*Psp—Src*), and 5.1% (*Emv-15—Src*), which confirm the order of these markers as determined from the long range restriction map and suggest that the physical distance between *Emv-15* and *Src* is similar to that between *Emv-15* and *Psp*.

D 501 THE COL11A2 GENE IS CLOSELY LINKED TO THE Aß3 GENE IN THE MOUSE MHC LOCUS.

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Collagens are major structural proteins of the extracellular matrix. Type XI collagen is a minor collagen component of hyaline cartilage, consisting of a heterotrimer of 3 different polypeptide subunits, α1(XI), α2(XI) and α3(XI). In man the COL11A1, and COL11A3 genes are located on chromosomes 1 and 12 respectively. The human COL11A2 gene is within 45kb of the DPB2 gene in the HLA locus on chromosome 6. In order to determine if the mouse COL11A2 gene is similarly linked to the histocompatibility complex in the mouse, the human gene was used to screen Southern blots prepared from BXD recombinant inbred mouse lines. These studies show that the mouse gene cosegregates with genes in the proximal portion of the mouse MHC on chromosome 17. In order to localise more precisely the mouse COL11A2 gene with respect to the MHC, a series of overlapping cosmids (gift of M.Steinmetz) were screened using the human gene as a probe. We have obtained a part of the mouse COL11A2 gene from a positively hybridising cosmid. The mouse COL11A2 gene shows nucleotide and amino acid homology of >90% in the region coding for the triple helical domain of the protein. The data suggest that the mouse and human COL11A2 genes are closely linked to the histocompatibility locus. The isolation of genes close to the major histocompatibility complex may provide tools for the study of diseases which are linked to this locus but not of histocompatibility origin. This study is supported in part by a Hong Kong University Strategic Research Grant.

D 502 THE INDIAN MUNTJAC: A COMPARATIVE GENE MAP, M.M. Cohen, H.P. Levy, R.A. Schultz, Div. of Human Genetics and Medical Biotechnology Center, University of Maryland, Baltimore, MD 21201

The Indian muntjac (Muntiacus muntjac) has the lowest known diploid mammalian chromosome number [female, 2N=6; male, 2N=7]. Flow cytometry demonstrated that muntjac cellular DNA content approximates 90% of the human. Its karyotype is well suited for development of a comparative gene map because of the chromosome number, the similarity in total genome size, and since individual muntjac chromosomes are so readily and unambiguously identified. Each muntjac chromosome has been flow-sorted to high purity (>98%) after staining with propidium iodide (for DNA content) and flouresceinated antikinetochore antibody (for centromere size). Chromosome-specific DNA extracted from these fractions, as well as muntjac and human total genomic DNA, has been prepared for Southern analysis. Flow-sorted muntjac chromosomes have also been applied directly to nylon filters as dot blots. Both Southern and dot blots are being hybridized with previously localized human cDNA probes. These genes (HGPRT, MIC2, ZFY, protein kinase C, XRCC, ERCC2, insulin) will identify apparently conserved regions of homology between the muntjac and human genomes. Previously unmapped human genes may first be localized with relative ease in the muntjac karyotype. Reference to the comparative map will then identify the human chromosomal region(s) homologous to the specific muntjac regions, thereby facilitating identification of the human locus.

D 503 MAPPING MOUSE ZINC FINGER PROTEIN GENES, Paul Denny and Alan Ashworth Cell and Molecular Biology Section, Chester Beatty Laboratories, Institute of Cancer Research, London SW3 6JB, United Kingdom.

The character and pattern of Drosophila segmentation is believed to be controlled by gene families which share particular DNA-binding motifs. By analogy, mammalian genes encoding these motifs, such as the homeo-box (Hox) and zinc-finger protein (Zfp) genes, may be involved in the establishment of segmental pattern. This process is first seen in the murine embryo at about day 8.5 (p.c.) and we have isolated cDNAs encoding zinc-finger proteins from a library representing this embryonic stage. Two of the genes (Zfp-3 and Zfp-4) encoding these proteins were mapped to specific regions of chromosomes 11 and 8, respectively (Ashworth et al (1989), Genomics 4: 323-327). The localisation of two other Zfp genes to these chromosomes and the precedents for the clustering of members of gene families suggested that these genes could be closely linked. We have therefore begun the long-range restriction mapping of these, and other Zfp genes, using pulsed-field gel electrophoresis (PFGE).

To provide clues as to the functions of these putative developmental control genes, we are analysing their patterns of transcription in adult and embryonic tissues. It is possible that their temporal or spatial pattern of expression will correspond to specific stages or structures in the process of segmentation.

D 504 A GENOMIC WALK ENCOMPASSING THE HUMAN Xq28 COLOR VISION PIGMENT GENES; SEARCH FOR DELETIONS IN ADRENOLEUKODYSTROPHY PATIENTS.

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A cosmid library was used to clone the red and green color pigment genes and flanking sequences. The constitution of a restriction map of this 195kb region allowed us to confirm that the color pigment genes are within very homologous units arranged in tandem array. 6kb upstream of the red pigment gene a NotI and a EagI site are present; the NotI site is unmethylated on the active X-chromosome and may represent a CpG island for the whole pigment gene cluster. Another CpG island , 61kb 3' of the last green pigment gene, is unmethylated on the active X-chromosome and flanked by conserved sequences. Aubourg et al. hypothesized the existence of rearrangements affecting both color vision and the ALD gene (Am. J. Hum. Genet. 1988; 42:408-413). Using six different probes from the cloned region we searched for deletions in 34 ALD patients. No deletions were found 3' of the pigment genes. A single ALD patient with blue monochromatic color vision was deleted for sequences 5' of the pigment genes suggesting that the ALD gene lies 5' of the red pigment gene.

D 505 Physical Localization of Not 1 and Eag 1 Boundary Clones on Chromosome 21.

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Libraries of Not 1 and Eag1 boundary clones were constructed in EMBL 6 from a somatic cell hybrid (725) containing Ch 21, plus a small integrated non-21 fragment. Human clones were identified by plaque hybridization with a total human probe, and selected clones were verified to be boundary clones by differences in their hybridization to Southern blots prepared from DNA restricted with Not 1 and Eco R1, versus Eco R1 alone. The clones (29 Not 1 and 38 Eag 1, each hybridizing differently on Southern blots) have been localized on Ch 21 with a mapping panel consisting of DNAs from 7 somatic cell hybrids with various deletions or translocation of Ch 21. The distribution of these clones is not random. In agreement with genes already mapped, our boundary clones show a marked preference to region q22. Thirty-one (46%) of the clones are located in band q22.3 which represents approximately 10% of the chromosome. Of interest, 12 clones have been assigned to part of the region of q22.3 bounded proximally by the acute myelogenous leukemia 8;21 translocation and distally by a 10;21 translocation. This region contains the D21S55 locus, which has been suggested by others to contain a minimal portion of Ch 21 whose trisomy is invariably associated with many of the phenotypic features of Down Syndrome. We are beginning to construct a pulsed field gel map of the region defined by these twelve boundary clones. This effort should be advanced by our recent construction of a Not 1 linking library from the 725 hybrid. We intend to use our boundary and linking clones from the proximal q22.3 region to probe cDNA libraries. It is anticipated that candidate genes so identified may subsequently be tested for their involvement in Down Syndrome. One experimental approach might be to corelate the overexpression of particular candidate genes in transgenic mice with phenotypic alterations similar to those found in individuals with Down Syndrome.

D 506 CHARACTERIZATION OF MURINE CD14: GENE STRUCTURE AND CHROMOSOMAL LOCATION.

Sanna M. Goyert, Chih-Lin Hsieh*, Uta Francke*, Enza Ferrero. Department of Medicine, Division of Molecular Medicine, North Shore University Hospital/Cornell University Medical College, Manhasset, N.Y. 11030 and *Howard Hughes Medical Institute and Department of Genetics and Pediatrics, Stanford University School of Medicine, Stanford, CA. 94305. We have isolated and characterized cDNA and genomic clones which encode the murine homolog of the human myeloid differentiation antigen, CD14. Northern analyses show that the expression of murine CD14 is similar to that of the human homologue in that it is restricted to hematopoietic cells of the myeloid lineage. Murine and human CD14 sequences are highly conserved with 74% nucleotide identity and 66% amino acid identity. In both species the protein sequence contains a repeating (10 times) leucine-rich motif (LXXLXLX) that is also found in several proteins from phylogenetically distant species including yeast adenylate cyclase, the drosophila proteins chaoptin and toll, the bovine and human proteoglycans PGI and PGII, the human serum α2 glycoprotein LRG, human placental RNase inhibitor and the human platelet membrane protein, GPIb. We have mapped the murine CD14 gene to chromosome 18. This chromosome contains at least five genes encoding receptors (Pdgfr, Adrb2r, Ii, Grl-1, Fms) that are part of a conserved syntenic group located on mouse chromosome 18 and human chromosome 5. The murine CD14 expression profile and chromosomal location further support our hypothesis that CD14 may function as a receptor.

D 507 ANALYSIS OF GENOMIC CLONES FROM THE X-LINKED MOUSE DYSTROPHIN LOCUS (*Dmd*). Andrew J. Greenfield, Mark K. Maconochie, Una L. Fairbrother and Stephen D.M. Brown. Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, London W2 1PG, U.K.

The X-specific mouse mutation *mdx* results in a muscular dystrophy of comparatively mild pathology, a phenotype caused by a premature termination codon in the gene for dystrophin. We have utilised selected DNA segments from the human dystrophin cDNA as hybridisation probes to isolate genomic clones from the cognate murine gene as a first step in determining the detailed structure of specific regions of the dystrophin locus and as a preliminary to creating targeted mutants at this locus in ES cells. We are investigating by sequence analysis a pcos2EMBL cosmid exhibiting homology to human 5' dystrophin cDNA sequences (exons 3-8) which segregates with *mdx* in a *spretus/domesticus* interspecific cross. In addition, we are examining several cosmid clones with varying homology to dystrophin cDNA, one of which maps to an autosomal locus on mouse chromosome 6; this work relates to the report of an autosomal dystrophin homologue in humans. Along with conventional cloning techniques, we are utilising fragment enrichment procedures to isolate sequences so far unclonable using cosmid vectors.

D 508 ANALYSIS OF THE STRUCTURE AND ORGANISATION OF AMPLIFIED DNA IN A RAT CELL LINE, Edith Heard and Mike Fried, Department of Eukaryotic Gene Organisation and Expression, Imperial Cancer Research Fund, P.O.Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Gene amplification, although not thought to be a normal event in mammalian cells, is seen to occur in tumour cells and transformed cell lines. Work in our lab. has shown that the structure of amplified DNA in various cell lines is in the form of arrays of inverted repeats. This was first demonstrated in a rat cell line (3B) containing an amplified polyoma oncogene. Long range mapping of the large inverted duplications in 3B using pulsed field gel electrophoresis has shown the amplification units are in excess of 400kb, and so far appear to be quite homogeneous. In order to find the extent of the units, to determine whether they are fully homogeneous, and to try and clone the other end of the inverted duplications Yeast Artificial Chromosome vectors have been used to try and clone large regions of the amplified DNA in 3B cells. In addition, *in situ* hybridisation of biotinylated probes from the amplified DNA onto chromosome spreads of rat cells has been used to determine the location of the amplified DNA.

D 509 AN UNMETHYLATED CpG RICH ISLAND IS LOCATED 4 KB DOWNSTREAM OF THE HUMAN LCAT GENE ON CHROMOSOME 16q22

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We have cloned human LCAT (lecithin cholesterol acyl transferase) cDNA and used it as a probe to isolate a cosmid clone containing a 38 kb genomic fragment. LCAT has previously been mapped to chromosome 16q22. The insert, with the complete LCAT gene in the 5' end, has an overabundance of sites for rare-cutting enzymes, indicating the presence of CpG rich islands, which often are found associated with genes. We have located one such island 4 kb downstream of the LCAT gene by sequencing. The area downstream of this island is also GC-rich and contains Alu-repeats. The island is shown to be unmethylated in white blood cell DNA by using methylation-sensitive restriction enzymes. A 0.5 kb part of the island hybridized to DNA from several mammals, shown by Southern blotting. This may indicate that we have located a conserved gene. Sequence homology search has so far not given any positive results. Another CpG rich region is located 8.5 kb further downstream.

D 510 Comparative Gene Mapping Using Monochromosomal Hybrids, Peter A. Lalley, and Julie Zelazny, Center for Molecular Biology, Wayne State University, Detroit, MI 48201. Comparative gene mapping studies in man and mouse can provide valuable information for understanding the organization and evolution of the mammalian genome and for developing animal models for human diseases. At least 43 conserved autosomal segments involving over 350 genes, 39 x-linked loci and 7 Y-linked loci have been identified between man and mouse. In order to define further the conserved regions in the mouse genome a series of monochromosomal hybrids each containing a single mouse chromosome or a fragment of a mouse chromosome are being developed. Random segregation of mouse chromosomes in somatic cell hybrids and microcell hybridization techniques together with x/autosome translocations and HAT selection or neomycin-resistant mouse clonal lines were employed. Using these approaches individual hybrid clones have been isolated containing mouse chromosome 2,5,13,15 and X respectively. In addition a hybrid clone containing the proximal two thirds of mouse chromosome 4, and three separate clones containing overlapping regions of mouse chromosome 7 have been isolated. Fifty independent neomycin-resistant clones have been isolated and are being used to develop other monochromosomal hybrids. These hybrids will provide an invaluable resource for producing high resolution physical maps and excellent starting material for producing chromosome specific libraries and for cloning any genes located on the chromosome or chromosomal fragment.

D 511 A CpG RICH ISLAND AT THE 5' END OF THE HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR GENE IS UNMETHYLATED

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Vertebrate DNA is generally depleted in the dinucleotide CpG, but many vertebrate genes contain CpG rich islands. There are about 30000 such islands in the murine genome. Typically, between 60 and 90 % of total CpGs are methylated, but in the CpG rich islands tested the cytosines are unmethylated. The human EGF receptor gene spans 110 kb of DNA, and the coding sequence is divided into 26 exons. We have analyzed a 4 kb Hind III clone from this gene, containing the first exon, 850 bp of noncoding 5' sequence, and a part of the first intron. In this fragment we have demonstrated the presence of a 2 kb CpG rich unmethylated island which includes the exon, by using methylation-sensitive restriction enzymes. The island is unmethylated in several different tissues. Sequencing of the clone showed that the island ends at a potential Z-DNA-forming poly(AC)-sequence. The clone is now being used in attempts to establish transgenic mice to study the methylation status of the island when introduced into the murine genome.

D 512 ISOLATION OF HUMAN SEQUENCES TRANSCRIBED IN INTERSPECIFIC SOMATIC CELL HYBRIDS, Randy J. Legerski, Paul Liu and Michael J. Siciliano, Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030. Human-rodent somatic cell hybrids are often highly reduced for human genomic content. The regions of the human genome remaining may be isolated by screening DNA libraries made from such hybrids for human specific repetitive sequences. While this is an important step in reverse genetic approaches to the cloning of biomedically important genes, subsequent identification of the genes themselves has proven problematic. The need for a method to directly isolate human transcribed sequences of expressed genes from such hybrids is therefore indicated. Making cDNA libraries from mature mRNA produced by such cells, though direct, would not achieve that purpose because the repeat sequences, needed to detect clones of human origin, are primarily located in introns which are removed during RNA message maturation. Here, after using consensus 5' intron splice sequences as primers to enrich for use of immature, unspliced mRNA (still retaining species-specific repeat sequences) as template for cDNA library construction, we report the direct cloning of human transcribed sequences from interspecific somatic cell hybrids. A library so made from a human x Chinese hamster cell hybrid, containing <1% of human genomic sequences, was screened with total human DNA. Of clones thus far studied and characterized all are human in origin and have single copy sequences which map to the regions of the human genome contained in the hybrid. Approximately 50% of the clones contain exons of transcribed human genes since they hybridize to discrete bands on Northern blots. (Supported in part by N.I.H. Grant CA 34936 and awards from M.D.A. and the Piton Foundation).

D 513 CONSERVATION OF THE MOUSE Tla GENE SUBFAMILY OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE MOUSE-LIKE RODENTS. Akihiro Matsuura, Shin-ichi Takayama, Ryo-ichi Honda and Kokichi Kikichi
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Mouse TL antigens are expressed on thymocytes and some T cell leukemias. Structural genes for TL(Tla) are located in the Tla region of the major histocompatibility complex and are related to class I genes (class Ib). Most of class Ib are considered to be redundant because neither genetic homologues had been yet found in any other species, nor any functions had been assigned. To adress the question whether Tla are conserved in evolution or just expanded specifically in mouse, we investigated several species of the rodent with a Tla specific probe derived from 3'region of a Tla gene,T13^c. While it detected a small subset of class I genes in the Tla region; four (T1^c,T3^c,T11^c,T13^c) of BALB/c and two (T1^b,T3^b) of B10 mouse, several hybridizing bands were obsereved in cellular blots of rat and hamster but not of guinea pig. To confirm the class I identity of homologous sequences, genomic clones were isolated and characterized from SD rat genomic library. There were at least three Tla-like genes in the rat. Partial nucleotide sequences of one of such clones indicated that exon 4 corresponding to $\alpha 3$ domain engaging the β_2M light chain was highly conserved. We therefore concluded mouse Tla genes were conserved at least in the mouse-like rodent (Myomorpha) and may have certain biological significances. We are now studying the structure and expression of rat Tla-like genes.

D 514 RFLPs AND LINKAGE RELATIONSHIPS OF THE HUMAN ACYL-COA DEHYDROGENASE GENE. N.J. Wandersee, W.J. Rhead, A.W. Strauss, K.H. Buetow, and J.C. Murray.
University of Iowa, Iowa City; Washington Univ. School of Medicine, St. Louis, Missouri and Fox Chase Cancer Center, Philadelphia, Pennsylvania.
Medium-chain acyl-CoA dehydrogenase (MCAD) is one of three principal enzymes involved in the first step in mitochondrial β-oxidation. MCAD is a homotetrameric enzyme; the gene for the MCAD monomer (ACADM) is located in the nuclear genome, and codes for a 50 kDa monomer that is cleaved to 46 kDa upon import into the mitochondrion. ACADM has been mapped to human chromosome 1, band p31, through the use of in situ hybridization and somatic cell hybrids. RFLPs for ACADM were identified by screening unrelated Caucasians with BamHI, BclI, BglII, EcoRI, EcoRV, HaeIII, HincII, HindIII, HinfI, KpnI, MspI, PstI, PvuII, SacI, StuI, RsaI and TaqI. Polymorphisms were identified with MspI (9 kb and 8kb alleles) and PstI (5kb and 3kb alleles). Inheritance was confirmed in 36 families for the MspI polymorphism and in 9 families for the PstI polymorphism. The Centre d'Etude du Polymorphisme Human (CEPH) DNA panels were investigated for linkage using RFLPs with ACADM (MspI), GLUT1, D1S21 (TaqI), D1S19 (SacI), D1S17 (TaqI), D1S18 (BglII), D1S22 (BclI), D1S2 (BglII), CRI-S182 (MspI), D1S16 (BglII), CRI-L816 (BamHI), CRI-L1201 (TaqI), D1S12 (XbaI) and with classical markers for RH (Rh antigen) and PGM1 (Phosphoglucomutase 1). Pairwise linkage analysis using the LINKAGE program MLINK established nine significant pairwise linkages ($Z \geq 3$) of ACADM with these markers. ACADM thus serves as a useful bridging marker on 1p, and as an addition to the map of the short arm of chromosome 1.

D 515 ONCOGENE COMPLEMENTATION IN HUMAN BREAST CANCER: AMPLIFICATION OF C-MYC, ERBB-2 AND INT-2 GENES, Magali Roux-Dosseto, Christine Desideri, Nadege Dussault, Sylvie Romain and Pierre M. Martin, URA CNRS 1175, Faculte de Medecine-Nord, 13326 MARSEILLE Cedex 15, FRANCE. Amplification of c-myc, erbB-2 and int-2 oncogenes has been investigated in tumor DNAs of 170 patients with primary breast cancer and concerns 27%, 28% and 17% of tumors, respectively. We further looked for any synergistic action of these genomic alterations on disease-free and overall survival. Using uni- and multivariate statistical analysis we showed that c-myc amplification was associated with early recurrence and shorter overall survival, in contrast erbB-2 and int-2 extra-copies resulted in later relapse events specially in patients whose tumors showed a normal copy number of c-myc genes. Co-amplified c-myc and erbB-2 genes showed positive cooperation with respect to disease reccurence and shortening of the overall survival. Finally, the harmful effects of amplified *c-myc and erbB-2 oncogenes were dramatically increased in patient subgroups* showing normal copy number of int-2 gene. A multivariate analysis was used to test for potential interactions of oncogene covariates. We pointed out multiple independant combinations which defined complementation groups with respect to clinical patient behaviour. As the large amount of DNA coamplified with the target genes may well contain other active transcription units, we are currently investigating the size and the organization of amplified regions *in tumor DNAs.*

D 516 Molecular characterization of the chromosomal segment spanning the Choroideremia gene at Xq21.

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Choroideremia (tapeto-choroidal dystrophy, TCD), an X-linked disorder of the retina and choroid, causes progressive nightblindness and central blindness in affected males.
Detailed molecular characterization of the X-chromosomal abnormalities in TCD-patients with and without other clinical features has enabled us to assign the TCD gene to a small segment centered around the DXS165 locus (Cremers et al., Genomics (1989) 4, 41 and Clin.Genet.(1987) 32, 421).
By using chromosome walking and jumping techniques combined with preparative-FIGE cloning methods we have isolated several clones from the TCD-relevant area at various distances from DXS165. Extensive physical fine mapping of seven microdeletions (collaboration with E-M. Sankila, A.Wright, F.Brunsmann) - one of which has a deletion of only 45 kbp - and one de novo 46,XX t(X;13) translocation associated with TCD (collaboration with W.Flinthoff) enabled us to
i) infer the physical order of several of these cloned DNA elements,
ii) identify the exact positions of various deletion end-points and the translocation breakpoint, iii) isolate several additional clones containing deletion-junction fragments by forced cloning of purified chromosomal DNA fragments and iv) define a common, 20 kbp large, region that overlaps all deletions but one. A restriction map of the entire TCD-area will be presented. Furthermore, we will report on the more detailed characterisation of a DNA segment of 45 kbp as well as several adjacent DNA segments that have been cloned from this area. Unique copy DNA elements isolated from this region fullfill at least two important criteria for being part of the candidate TCD-gene, in that they are evolutionary conserved and display homology to mRNA sequences isolated from retina.

D 517 MAPPING THE BREAKPOINTS IN STEROID SULFATASE DELETION PATIENTS USING S232 SEQUENCES.

P.H. Yen, X.-M. Li, S.-P. Tsai and L.J. Shapiro. Howard Hughes Medical Institute Laboratories and Departments of Pediatrics and Biological Chemistry, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509. X-linked ichthyosis, caused by a deficiency of steroid sulfatase activity, is one of the most common human genetic diseases affecting one in 2-5 thousand males. Over 90% of the patients have deletions of their entire steroid sulfatase gene located on the distal short arm of the human X chromosome. Results from several labs suggest that deletions at the STS locus are of substantial size. Mapping and sequencing the breakpoints may reveal possible mechanism(s) of the frequent deletions at this locus. To this goal we have used the DNA clone CRI-S232 (NAR 17:423, 1989) which detects sets of highly polymorphic restriction fragments on the X chromosome as well as non-polymorphic fragments on the Y. Southern hybridization using S232 as a probe showed that DNA's from the 23 deletion patients all gave fewer bands than normal controls. To simplify the analyses we have isolated clones containing S232 sequences from a human cosmid library. Single copy sequences were isolated from these clones and mapped on the X and Y chromosomes. We found that there are at least four copies of S232 sequences on the X, one distal (CS17) and three proximal (CS12, CS21 and CS19) to the STS locus. These S232 sequences are on different Sfi I fragments, ranging from 100 kb to 800 kb. Most of the patients retain two of the proximal copies, with the breakpoints occurring between clones CS12 and CS21, at least 600 kb 3' of STS. A probe from CS21 detects an altered Sfi I fragment in several patients. This probe will facilitate our cloning of the breakpoints. In addition, we are also studying the mechanism that generates the polymorphism detected by S232.

D 518 MOLECULAR CLONING AND CHARACTERIZATION OF A HUMAN X-LINKED GENE, A1S9, COMPLEMENTING A TEMPERATURE-SENSITIVE, S PHASE MUTANT OF MOUSE L-CELLS.

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Previous analysis of the ts A1S9 mouse L-cell mutant revealed that it is defective in a gene required for nuclear, but not polyoma viral DNA replication. In addition, both active and inactive X chromosomes can correct the ts A1S9 lesion in cell hybrids, suggesting that the gene escapes X inactivation. We have cloned the human A1S9 gene by complementing the ts A1S9 cell defect with human DNA and subsequentely retrieving the human DNA region (42 kbp) retained in independent transformants. We identified a unique fragment, derived from the A1S9 locus and conserved in independent transformants which recognizes a 3.6 kb poly (A^+) mRNA. This probe was used to isolate several partial cDNA clones. Preliminary sequence analysis and search in a DNA sequence databank suggests that A1S9 is a novel gene. Using Southern and *in situ* hybridizations, we have assigned the A1S9 gene to Xp11.2-11.4. Quantitative Northern Blot analysis does not reveal an increase in expression of the A1S9 gene in diploid human cell lines harbouring increasing numbers of X chromosomes. Thus, the A1S9 gene may not escape inactivation but may be derepressed at high frequency following the fusion of human and ts A1S9 cells. We are testing this hypothesis by analysing human-mouse hybrids containing human inactive X chromosomes.