GENE TRANSFER AND GENE THERAPY

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Page

Plenary Sessions

February 7:
Model Systems for Gene Transfer 159
Gene Transfer - I 160

February 8:
Gene Transfer - II 161
Gene Transfer - III 161

February 9:
Gene Transfer - IV 163
Human Genetic Diseases - I 163

February 10:
Human Genetic Diseases - II 165
Human Gene Therapy 166

February 11:
Tissue Specific Enhancers 167
Recombination 168
Late Addition 169

Poster Sessions

February 7:
Vectors and Gene Transfer 170

February 8:
Transgenic Animals 186

February 11:
Recombination, Enhancers and Genetic Diseases 196

Gene Transfer and Gene Therapy

Model Systems for Gene Transfer

H 001 TRANSGENIC FLIES AND THE CONTROL OF SEXUAL DIFFERENTIATION BY ALTERNATIVE SPLICING, Michael McKeown[1], John M. Belote[2] and Russell T. Boggs[1], 1. Molecular Biology and Virology Laboratory, The Salk Institute,PO Box 85800,San Diego, CA 92138 , 2. Department of Biology, Biological Research Laboratory, Syracuse University, 130 College Place, Syracuse, NY.13210

The *transformer* (*tra*) gene is one of the genes of the sex determination hierarchy in Drosophila. These genes function to control sexual differentiation via control of the bifunctional *doublesex* (*dsx*) locus. Genetic studies indicate that information about the primary signal for sexual differentiation (the X chromosome to autosome ratio, X:A) is transmitted through the genes of this hierarchy in the order: X:A →*Sex lethal* (*Sxl*) →*transformer,transformer-2* (*tra-2*) →*doublesex*. In females *Sxl*, *tra* and *tra-2* are active and cause *dsx* to be expressed in its female mode. In males *Sxl*, *tra* and *tra-2* are not active and *dsx* is expressed in its basal, male mode. An additional gene, *intersex*(*ix*), is necessary for *dsx* female function.

tra has been cloned and shown to give rise to two RNAs. One of these is about 0.9 kb and is present only in females. It induces female differentiation. The other is about 1.1 kb and is present in both sexes. It is apparently without function. In order to determine the relationship between and the regulation of the two *tra* RNAs, and the relation of these RNAs to the neighboring genes, we have used S1 mapping, primer extension and sequencing of genomic and cDNA clones for *tra* and both of the adjacent genes. 80 bases separate the 3' end of the upstream RNA from the start of the *tra* transcription unit. This extends for just over 1 kb. The 3' end of *tra* overlaps the 3' end of the adjacent gene by about 70 bases. The non-sex-specific *tra* RNA contains two small introns. The female-specific RNA is similar but uses an alternative splice acceptor (175 bases farther downstream) for the first intron. The non-sex-specific RNA has no long ORF while the female RNA has a single ORF starting with the first AUG and extending until just short of the overlap at the 3' end. Thus, an RNA present in an unexpected tissue is apparently noncoding. We have shown that regulation of *tra* is promoter-independent by replacing the *tra* promoter with a heat shock promoter. Otherwise *tra*⁻ females carrying this construct show substantial female differentiation, especially after heat shock.

The sex-specificity of RNA patterns has allowed us to test, at the RNA level, the regulatory interactions inferred from genetics. Our studies show that *Sxl*⁺ activity is necessary for *tra* female activity. XX; *tra-2*⁻ flies develop with full male morphology and yet express *tra* in its female mode, indicating that *tra-2* does not regulate *tra* and must either be below it in the hierarchy or on a separate branch. This also indicates that *dsx* male does not inhibit *tra* female expression and that *dsx* female is not necessary for *tra* female expression ie. there is not a feedback loop. In agreement with these inferences, XX; *dsx*⁻ and XY; *dsx*⁻ flies express *tra* in a manner consistent with their chromosomal sex, indicating that *dsx* does not regulate *tra* . In order to determine if *tra* and *tra-2* are in a linear or branched order, and to determine if there are any other sexually regulated genes acting outside the known regulatory path, we have constructed a heat shock-*tra*-female fusion which constitutively expresses the *tra* female RNA. XY flies carrying this construct develop as females, indicating a linear order of gene action and showing that other genes such as *ix* must be under the control of *tra* if they are regulated in a sex-specific manner. We have demonstrated that the *tra* male RNA has little or no function by producing flies deleted for most or all of the *tra* gene. Males homozygous for this mutation are phenotypically normal, viable and fertile. XX or XY flies which are deleted for *tra* but which carry the hs-*tra*-female cDNA develop as females.

H 002 Expression of Genes Coding for Proteins Involved in Synthesis and Binding of Epinephrine. Richard Palmiter, Edward Baetge, James Allen, Richard Behringer* and Ralph Brinster*. Howard Hughes Medical Institute, University of Washington, Seattle WA and *School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA.

The human gene coding for the last enzyme in catecholamine biosynthesis, phenylethanolamine N-methyl transferase (PNMT), that is responsible for the synthesis of epinephrine in the adrenal medulla and a small subset of neurons within the brain stem and neural retina, was isolated and characterized. When this gene was introduced into the germline of mice, mRNA expression was detected predominantly in adrenals and eyes. Immunostaining was used to show that when the 5' flanking region of PNMT was fused to SV40 T-antigen, expression was limited to the medullary cells of the adrenal. At birth, the adrenals were of normal size and function but there was a gradual hyperplasia that culminated in adrenals the size of kidneys by 3 months of age. Adrenal growth was associated with increased output of norepinphrine and dopamine into the circulation and consequent increase in blood pressure. Many of these mice also manifest hyperplasia of cells within the ganglion and inner nuclear layers of the retina. By introducing a gene containing the 5' flanking region of PNMT joined to diphtheria toxin A, we have generated transgenic mice in which the adrenal medullary cells are ablated during development. These mice survive despite the lack of circulating epinephrine, although they appear emaceated. We suspect that the absence of PNMT-positive neurons, that are thought to be involved in regulation of hypothalamic functions, is responsible for the gaunt phenotype. We have also cloned the mouse ß2-adrenergic receptor gene and have shown that it produces a functional receptor when introduced into adrenalcortical Y1 cells that normally lack these receptors. After gene transfer, these cells bind ß-agonists such as isoproterenol with a Kd of 5 nM and this binding activates adenylate cyclase and steroid biosynthesis. These cells become unresponsive to continuous agoinst exposure after a few hours. We are currently attempting to direct high level expression of this receptor to cells of transgenic mice that normally have ß2 receptors (such as hepatocytes) as well as to cells that lack these receptors (pancreatic acinar cells). This might allow experimental manipulation of cAMP levels in specific cell types under *in vivo* conditions.

Gene Transfer and Gene Therapy

Gene Transfer - I

H 003 LARGE DNA TECHNOLOGY AND POSSIBLE APPLICATIONS IN GENE TRANSFER. Charles R. Cantor and Cassandra L. Smith, Department of Genetics and Development and Departments of Microbiology and Psychiatry, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

It has now become fairly routine to analyze DNA on the megabase size scale by using pulsed field gel electrophoresis (PFG). In simple organisms this technique can provide an overview of the entire genome at a size resolution of 5 kb. Thus any DNA insertion or deletion of this magnitude or any other rearrangement causing such an alteration in a macrorestriction fragment can be detected without the need for cloned DNA probes. In mammalian genomes, under optimal conditions, discrete macrorestriction fragments can be visualized in an entire genomic digest but it is not yet known whether the bands seen can be used for direct analysis, since they probably represent a large set of overlapping fragments. However, with appropriate repeated DNA or pooled DNA probes, it is possible to detect and analyze tens of megabases of DNA simultaneously. Examples of this will be demonstrated for human chromosomes in hybrid cell lines, for gene families with crosshybridizing probes, and for selected low copy number repeated sequences. PFG should be useful in monitoring the results of gene transfer wherever the size and distribution of multiple insertions is of interest or wherever it is desirable to examine rearrangements of a DNA region that may accompany or follow DNA insertion.

H 004 VACCINIA VIRUS VECTORS, Bernard Moss and Thomas R. Fuerst, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Vaccinia virus is a large DNA virus that replicates in the cytoplasm of a variety of avian and mammalian cells. In tissue culture, the virus usually replicates over a 2 to 3 day period during which host cell specific macromolecular synthesis is turned off. In susceptible animals, vaccinia virus typically causes a local infection that lasts 2 to 3 weeks. Plasmid vectors, carrying vaccinia promoters, have been designed to facilitate the construction and selection of live recombinant viruses. Because of the large capacity of the vaccinia genome, multiple genes may be expressed simultaneously. A hybrid bacteriophage T7/vaccinia virus expression system has been developed. In this system, the T7 RNA polymerase is expressed by vaccinia virus and the phage enzyme is used to transcribe genes containing T7 regulatory sequences. Vaccinia virus vectors have been used to study protein synthesis, processing and transport; to prepare large amounts of protein in cultured cells; to determine the targets of humoral and cell mediated immunity; and as candidate live recombinant vaccines.

Gene Transfer - II

H 005 DESIGN OF RETROVIRUS VECTORS FOR GENE TRANSFER, A. D. Miller, M. A. Bender, M. A. Adam and R. E. Gelinas, Department of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.
We have investigated several parameters that are important in the design of retroviral vectors for gene transfer. It is clear from many studies that high titer virus is crucial for efficient infection of cells, especially for infection of normal human somatic cells. Using retroviral vectors carrying different selectable markers, we have shown that the signal required for efficient packaging of viral RNA into virions is larger than previously defined (Psi) and extends into the gag region of murine retroviruses. Inclusion of the complete signal (Psi$^+$) in retrovirus vectors followed by transfer of the vector into retrovirus packaging cell lines results in vector titers that are equivalent to those of wild type replication-competent viruses. Inclusion of the Psi$^+$ signal in a non-retroviral RNA results in packaging of the hybrid RNA into virions with high efficiency compared with retroviral vectors containing the signal, and packaging is dependent on proper orientation of the signal with respect to transcription. These studies define the extent of the packaging signal required for efficient packaging of retroviral RNA into virions.

We have also studied factors that influence the production of high-titer vectors carrying a gene with introns, specifically the human beta-globin gene. A variety of difficulties were encountered with vectors containing this gene, which could be partially but not completely resolved. In particular, a region in the second intron of the gene was found to be necessary for expression of the gene in erythroid cells, but which also inhibited virus production. Various modifications of the vector that resulted in relatively high titer vector production will be discussed.

Gene Transfer - III

H 006 PROGRAMMED AMPLIFICATION AND ABLATION OF SPECIFIC CELL LINEAGES IN TRANSGENIC MICE. Alan Bernstein, Siu Pok Yee, Alain Lavigueur, Janet Rossant, Martin Breitman and Tony Pawson. Mt. Sinai Hospital Research Institute, Toronto, Canada.
By introducing oncogenes or a toxin gene into the mouse germ line, we have been able to amplify or ablate specific cell types using tissue-specific gene regulatory elements. Three such examples will be described. Mice carrying the v-fps onc gene driven by the human β-globin/3' enhancer have a complex phenotype, including a shaking syndrome, cardiomegaly, early onset of a variety of malignant and benign neoplasms, and vascular abnormalities. Some of these effects have been shown to be dependent on the presence of the globin 3' enhancer. Mice carrying a p53 gene cloned from Friend erythroleukemia cells express very high levels of p53 in all tissues examined. Approximately 10% of these animals develop aggressive tumors, including osteosarcomas, thymomas, and carcinomas, by 4-6 months of age, demonstrating unequivocally the oncogenic potential of the p53 gene. Animals carrying the diphtheria toxin A (DT-A) chain gene driven by the lens-specific mouse γ_2-crystallin promoter develop micropthalmia and have lenses grossly deficient in fiber cells. These mice transmit both the γ_2DT-A gene and the micropthalmic phenotype will full penetrance, demonstrating that ablation events can be heritably programmed.

H 007 EXPRESSION IN HAEMOPOIETIC CELLS OF GENES DELIVERED BY RETROVIRAL VECTORS, Suzanne Cory, D.D.L. Bowtell, T. Gonda, and G.R. Johnson, Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

We have compared several recombinant retroviruses for gene delivery to murine haemopoietic cells both in vivo and in vitro. Viruses bearing only a neo^R gene were relatively efficiently expressed in infected cell lines and also in haemopoietic tissues of long-term reconstituted mice. The level of expression was significantly greater for proviruses carrying a modified LTR in which the Moloney enhancer had been replaced with that from the myeloproliferative sarcoma virus. Viruses encoding neo^R and designed to express an additional gene from the viral genomic RNA were expressed poorly in vitro and undetectably in vivo (1). With these results in mind, we decided to forgo the advantage of G418 selection and replace the neo^R gene in a simple vector by the gene of interest. The results obtained with these Zen viruses carrying several different genes will be discussed, particular emphasis being given to that encoding the granulocyte-macrophage colony stimulating factor (GM-CSF) gene.

1. Bowtell, D.D.L., Johnson, G.R., Kelso, A. and Cory, S. (1987). Mol. Biol. Med. 4 in press.

H 008 ONCOGENE EXPRESSION IN TRANSGENIC MICE AND MURINE STEM CELLS, Erwin F. Wagner, Ulrich Rüther, Colin L. Stewart, R. Lindsay Williams and Gordon Keller*, EMBL, D-6900 Heidelberg, F.R.G. *Basel Institute of Immunology, CH-4005 Basel, Switzerland.

Oncogenes are being introduced into embryos, embryonic stem (ES) cells and bone marrow cells to study their role in growth control and differentiation. To analyze the function of the proto-oncogene *c-fos in vivo,* various constructs carrying the *c-fos* gene under the control of an inducible or a constitutive promoter were microinjected into fertlized eggs and several transgenic mouse lines were established. The deregulated expression of exogenous *c-fos* genes specifically interferes with normal bone development, despite expression in other organs. However, high expression of *c-fos* in hemopoietic organs seems to interfere with the differentiation of lymphoid cells.

In a different experimental approach, retroviral vectors carrying a selectable gene together with a viral oncogene such as *v-src* or Polyoma middle T antigen are being used for infection of embryos, ES cells and bone marrow cells. Recent data from *in vitro* and *in vivo* experiments will be presented, which should help to understand the role of oncogenes in normal and malignant cells.

Gene Transfer - IV

H 009 FATE AND GENE EXPRESSION IN RETOVIRALLY-INFECTED CELLS GRAFTED TO THE RAT BRAIN. Friedmann, T.+, Shinohama, S.*, Fagan, A.*, Rosenberg, M.+, Wolff, J.+ and Gage, F.* UCSD School of Medicine, Departments of Neurology* and Pediatrics+, La Jolla, CA 92093.

A variety of rat cells infected in vitro with retroviral vectors and expressing the human HPRT, firefly luciferase and E.coli β-galactosidase (lac Z) reporter genes have been implanted into regions of the rat brain and the survival and proviral expression of the cells have been studied. The stability of the implanted cells is variable, with graft survival extending from several weeks to several months. Most grafts become well vascularized and show both glial and macrophage responses but little or no lymphocytic infiltration. Proviral expression in the absence of selection is also highly unstable, and is correlated partly with in vitro evidence of proviral instability in the absence of selection. These studies indicate that genetically modified cells implanted into the mammalian CNS may survive and express a proviral gene function for prolonged periods of time. Present efforts are being made to prolong cell survival and to identify the mechanical, immunological, genetic and other factors responsible for cell disappearance and provirus shutdown.

Human Genetic Diseases - I

H 010 MOLECULAR GENETIC STUDIES OF CYSTIC FIBROSIS, Arthur L. Beaudet, Gerald L. Feldman and William E. O'Brien, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030

Cystic fibrosis (CF) is the most common lethal genetic disorder in Caucasians. Physiological evidence suggests a defect in regulation of chloride channels in epithelial cells from sweat glands, respiratory tract, and perhaps intestine and pancreas. Collaborative linkage analysis has mapped the CF locus to chromosome 7q22 approximately 1 centimorgan from the met oncogene and from the D7S8 locus. Many laboratories are attempting to identify DNA clones for development of a physical map of the region surrounding the CF locus with the goal of cloning the gene. We have focused on the use of a somatic cell hybrid containing a small portion of chromosome 7 for isolation of clones. Recently Estivil et al. (1) identified a region of DNA which shows striking linkage disequilibrium with the CF mutation. We have used the XV-2c and KM-19 probes and have confirmed strong linkage disequilibrium in a North American population (2). The B haplotype (1 allele for XV-2c and 2 allele for KM-19) was found for 160 of 186 CF chromosomes, while the B haplotype occurred in only 27 of 180 normal chromosomes. This linkage disequilibrium indicates that most or all of the mutant CF genes in the population are derived from a single mutational event. The data suggest that direct detection of the CF mutation at a DNA level should allow for the development of heterozygote screening programs in the general population, although it is uncertain what proportion of the population would choose to avoid the disease by altering reproductive behavior.

We have performed molecular prenatal diagnosis in over 90 pregnancies at one in four risk. DNA analysis for prenatal diagnosis of CF is informative in at least 98% of families using very tightly linked DNA markers. We are not aware of any diagnostic errors using DNA analysis, although one crossover between XV-2c and KM-19 suggests that the CF mutation lies to the KM-19 side of XV-2c. DNA analysis and linkage disequilibrium can be used for prenatal diagnosis in families with less than one in four risk of CF.

It is estimated that there are between 25,000 and 30,000 living patients with CF in the United States. If gene therapy is to be relevant to cystic fibrosis, it may be necessary to correct the defect in respiratory epithelial cells which appear to cause the major morbidity and mortality.

1. Estivill, X., Farrall, M., Scambler, P. et al. (1987) Nature 326:840-845.
2. Beaudet, A., Spence, J., Montes, M., O'Brien, W., Estivill, X., Farrall, M., Williamson, R. (1987) N Engl J Med, in press.

H 011 MOLECULAR GENETICS OF FAMILIAL HYPERCHOLESTEROLEMIA, Joseph L. Goldstein, M.D. and Michael S. Brown, M.D., The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235

Familial Hypercholesterolemia is a common genetic disease that affects one in 500 individuals in the heterozygous form. Such heterozygotes have elevated levels of plasma cholesterol and an increased risk for atherosclerosis beginning at age 35. The basic defect in FH is due to a series of allelic mutations that disrupt the function of the low density lipoprotein (LDL) receptor locus. The LDL receptor mediates the removal of cholesterol-carrying lipoproteins from blood and thus controls the plasma cholesterol level. When the activity of LDL receptor is reduced as in FH heterozygotes, LDL builds up in plasma and deposits in the artery wall, thus causing atherosclerosis.

Cloning, DNA sequencing, and polymorphism studies of mutant LDL receptor genes isolated from FH subjects have shown that the disease is heterogeneous at the molecular level. At least 16 allelic mutations have been characterized at the DNA sequence level. In general, each affected family with FH from the U.S. and Western Europe has a different mutation. The frequency of FH in certain genetically isolated populations, such as Lebanese Arabs and French Canadians, is higher than that in the general population of the U.S. and Western Europe. We have shown that a founder gene effect is responsible for the increase incidence of FH in these groups. A deletion that removes the promoter of the LDL receptor gene occurs in the majority of FH subjects in the French Canadian population. A nonsense mutation in codon 660 of the receptor mRNA is the underlying cause of most FH in persons of Lebanese descent.

One striking finding that has emerged from the analysis of gross rearrangements in the gene for the LDL receptor is the involvement of middle repetitive Alu sequences in the generation of human mutations. Alu sequences are found at recombination junctions in at least five different FH genes, including both deletion and duplication mutations.

These studies, and recent studies on the expression of the human LDL receptor in transgenic mice, will be discussed.

H 012 MEMBRANE PROTEIN 4.1 GENE AND HEMOLYTIC ANEMIA, John G. Conboy, Narla Mohandas, Jeff Chan and Yuet W. Kan. Cancer Research Institute, Department of Medicine and Howard Hughes Medical Institute, University of California, San Francisco, CA 94143.

Red cell membrane protein abnormalities result in a class of hemolytic anemias characterized by abnormal red cell morphology such as spherocytosis or elliptocytosis. We have been studying a type of elliptocytosis associated with defects in the membrane protein 4.1. Protein 4.1, a major component of the membrane skeleton, stabilizes the spectrin-actin network and helps bind the membrane skeleton to the lipid bilayer through its interaction with transmembrane proteins band 3 and glycophorin. We have previously cloned the cDNA from this protein and studied its expression in families with hereditary elliptocytosis. In one family, there is a rearrangement of the gene in the region of the initiation codon of the mRNA which results in absence of protein 4.1. In another, an elongated protein 4.1 contains a corresponding elongated mRNA, most likely due to abnormal splicing.

The erythrocyte protein 4.1 cDNA from normal individuals shows evidence of alternative splicing. We have isolated from a reticulocyte cDNA library several forms of cDNA which differ by the addition or deletion of 21, 43, or 34 amino acids. In another variation, deletion of 80 nucleotides near the 5' end of the mRNA creates a new open reading frame 5' to the normal initiation codon, thus extending the amino terminus by about 170 amino acids. By Northern blot analysis, protein 4.1 is found to be expressed in all tissues studied. The length distribution of the mRNA varies in different tissues, suggesting that the distribution of these alternative splicing sites differs in different tissues, and that each isoform may serve a different function.

Human Genetic Diseases - II

H 013 MOLECULAR BASIS OF OSTEOGENESIS IMPERFECTA (OI), Peter H. Byers, Daniel H. Cohn,
Barbra J. Starman and Marcia C. Willing, Departments of Pathology and Medicine and Center of Inherited Disease, University of Washington, Seattle WA 98195

Osteogenesis imperfecta is a heterogeneous group of generalized connective tissue disorders, characterized primarily by bone fragility, that results from mutations in the genes encoding the chains of type I collagen. On the basis of clinical phenotype, four types of OI have been discriminated: OI type I, a mild, dominantly inherited disorder of bone fragility, blue sclerae and normal stature; OI type II, lethal in the perinatal period and usually resulting from new dominant mutations; OI type III, the genetically heterogeneous progressive deforming variety; and OI type IV, characterized by moderate short stature, bone fragility and normal sclerae.

Linkage studies in families with the OI type I phenotype indicate it o be genetically heterogeneous but most commonly linked to polymorphisms in the COL1A1 gene. Fibroblastic cells from these individuals synthesize about half the normal amount of type I collagen, usually the result of synthesis of half the normal amount of proα1(I) chains. Deletion of the entire COL1A1 gene appears to be rare and the majority of mutations that produce the phenotype are likely to affect splicing of the complex mRNA precursor. The OI type II phenotype usually results from point mutations that result in substitution for single glycine residues within the triple-helical domain of the proα1(I) chain and occasionally (less than 5% of the time) results from rearrangements within the COL1A1 or COL1A2 gene. These mutations result in synthesis of an unstable protein that is inefficiently secreted and interferes with normal molecular interactions when secreted. Because the COL1A1 gene is the usual site of mutation 75% of all type I collagen molecules are affected. The OI type III phenotype is also genetically heterogeneous. In one instance, homozygosity for a frame-shift mutation that alters the carboxyl-terminal sequence of proα2(I) chains and interferes with the incorporation of the chains into molecules, results in secretion of type I procollagen molecules that contain only proα1(I) chains. In other families, point mutations in the COL1A1 and COL1A2 genes that interfere with triple-helix stability result in the phenotype. OI type IV generally results from mutations (including point mutations and small deletions) in a COL1A2 allele although rarely point mutations in the COL1A1 gene produce the same phenotype. The phenotypic effect of mutations in the genes encoding the chains of type I procollagen depends on the nature and location of the mutation.

H 014 MOLECULAR GENETICS OF BETA-HEXOSAMINIDASE. Elizabeth F. Neufeld, Dept of
Biological Chemistry, University of California, Los Angeles CA 90024.

The lysosomal enzyme, β-hexosaminidase, has two subunits arranged in three isozymes: A(αβ), B(ββ), S(αα). The subunits are extensively processed post-translationally (1). The α- and β-polypeptides and the genes in which they are encoded have structural similarity, for which a common evolutionary origin has been suggested (2-4). Mutations in the α-chain gene (Tay-Sachs disease) and in the β-chain gene (Sandhoff disease) are heterogeneous; they include deletions and non-deletions (5,6). A defect in mRNA processing is suspected in the Ashkenazi Tay-Sachs mutation because of profound mRNA deficiency (2,3) in spite of quantitatively normal transcription (7). Other mutations interfere with αβ association (8), catalytic activity (9) or transport of the polypeptide from the endoplasmic reticulum (10). The latter group includes mutations that result in a truncated α-chain and an extended β-chain.

(1) Little et al, JBC, in press; (2) Myerowitz et al, PNAS 82, 7830, 1985; (3) Korneluk et al, JBC 261, 8407, 1986; (4) Proia, PNAS, in press; (5) O'Dowd et al, JBC 261, 12680, 1986; (6) Myerowitz and Hogikyan, JBC 262, 15396, 1987; (7) Paw and Neufeld, JBC, in press; (8) d'Azzo et al, JBC 259, 11070, 1984; (9) Ohno and Suzuki, J Neurochem, in press; (10) Zokaeem et al, AJHG 40, 537, 1987.

H 015 THE DUCHENNE MUSCULAR DYSTROPHY LOCUS: A 2000 kb GENE WITH A 400 kDa PROTEIN, Ronald G. Worton, Arthur H.M. Burghes, Elizabeth E. Zubrzycka-Gaarn, Dennis E. Bulman, Henry J. Klamut and Peter N. Ray, Department of Genetics and Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Duchenne muscular dystrophy (DMD) and its milder form, Becker muscular dystrophy (BMD) are allelic X-linked muscle wasting disorders. The responsible gene has been recently cloned from knowledge of its map location at band Xp21 in the short arm of the X chromosome. The cDNA recognizes a 14 kb transcript from fetal and adult skeletal muscle, as well as from fused myotubes in myogenic cell cultures. In total the cDNA probe recognizes over 60 Hind III bands on genomic digests of human DNA indicating a minimum of 60 exons in the gene. One or more exons are deleted in over 50% of boys with DMD or BMD and several translocation exchange points in females with the disease map within the gene. The total gene size is estimated at 2000-3000 kb. The size of the transcript predicts a 400-450 kDa protein product. In collaboration with J. Talbot and R. Hodges (University of Alberta) antibodies specific for the N-terminal region of the DMD gene product have been generated by immunization of rabbits with synthetic peptides based upon the cDNA sequence data at the 5' end of the gene. A second set of antibodies specific for the same region have been generated using as immunogen, fusion protein produced from cDNA subcloned into two different fusion vectors. By Western blot analysis, these antibodies recognize the fusion product as well as a 400 kDa protein from adult skeletal muscle. In studies of cultured muscle, the antibodies label the cell surface of fused myotubes. In collaboration with G. Karpati (Montreal Neurological Institute) an avidin-biotin based immunoperoxidase technique on cryostat sections of human muscle biopsies has revealed specific labelling of the plasma membrane (sarcolemma). The DMD gene thus seems to encode a large protein component of the muscle cell membrane. The function remains to be elucidated.

Human Gene Therapy

H 016 USE OF RETROVIRAL VECTOR-MEDIATED GENE TRANSFER TO OBTAIN EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN MURINE HEMATOPOIETIC CELLS, C. Thomas Caskey, Grant R. MacGregor, Frederick A. Fletcher, Kateri A. Moore and John W. Belmont, Howard Hughes Medical Institute, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.
Sixteen replication-defective retrovirus vectors which carry human adenosine deaminase (ADA) coding sequences were constructed to evaluate several functional parameters: (a) extended viral *gag* gene sequences in packaging of defective particles; (b) internal promoters in independent transcriptional units; and (c) altered 3' LTR's in packaging and expression. Each vector was transfected into the amphotropic packaging cell line PA317 or the ecotropic packaging cell line ψ_2 and stable transformant populations were tested for their ability to transduce ADA to Rat 208F target cells. Virus containing supernatants from populations which were positive in the initial screen were used to infect the ecotropic packaging cell line ψ_2. Individual ψ_2 clones were then tested for ADA transduction. These studies indicated that optimal production of virus was dependent on the region of *gag* 3' to the classical ψ packaging sequence. Vectors with altered 3' LTR's function poorly in the production of virus.

Retrovirus vectors with LTR, *cFos*, and HSVTK infect mouse primary bone marrow cells. Virus transduction of the human ADA was assessed in *in vitro* colony forming cells (CFU-C) and spleen colonies (CFU-S) using enzyme assay and Southern analysis. Human ADA was expressed in progenitors infected with the viruses having LTR and HSVTK promoters but not in those with the *cFos* promoter. Seventeen of nineteen mice injected with a fully reconstituting dose of infected marrow showed expression of human ADA in their peripheral blood after 8 weeks. By 12 weeks, however, a substantial reduction of ADA expression was noted. The mechanism of the reduction is under investigation. These studies provide preliminary basic data on the feasibility of gene transfer as a therapy for ADA deficiency.

H 017 VIRAL TRANSDUCTION AND EXPRESSION OF HUMAN PHENYLALANINE HYDROXYLASE IN MOUSE PRIMARY HEPATOCYTES, Savio L.C. Woo, H. Peng, D. Armentano, G. Darlington, and F.D. Ledley. Howard Hughes Medical Institute, Dept. of Cell Biology, Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.

Deficiency of phenylalanine hydroxylase (PAH) results in phenylketonuria (PKU) which causes severe mental retardation in untreated children. The disorder is transmitted as an autosomal recessive trait and has a prevalence of about 1 in 10,000 births among Caucasians. The enzyme is expressed only in the liver and requires tetrahydrobiopterin as a cofactor for activity. The cofactor is oxidized to the dihydro-form and must be recycled to the tetrahydro-form by dihydropteridine reductase which is preferrentially expressed in the liver. To explore the feasibility of somatic gene therapy for PKU, a human PAH cDNA was cloned into the N_2 retroviral vector. In order to target the gene for expression in cells of hepatic origin, expression of human PAH cDNA in this construct is under the control of the transcriptional regulatory element of the human α1-antitrypsin gene which is abundantly expressed in the liver. This promoter element is only several hundred base pairs in length and its in vivo activity has been demonstrated by its ability to drive the expression of a reporter gene in the livers of transgenic mice. This recombinant was introduced into ψ2 and PA317 packaging cell lines to produce recombinant viruses of $5X10^5$ CFU/ml. Primary hepatocytes were isolated from neonatal mouse and plated in defined hormone medium without serum. The cells not only retain hepatic morphology and function, but are also capable of undergoing several rounds of nuclear division. The cells were infected with recombinant viruses and selected with G418. DNA analysis from the G418-resistant hepatocytes indicated that the recombinant virus was integrated into the cellular genome and RNA analysis showed the presence of a high level of human PAH RNA in the infected mouse cells. The results demonstrated the feasibility of transducing functional genetic material into primary mouse hepatocytes by retroviral-mediated gene transfer and provide the basis for futher investigation towards the ultimate goal of somatic gene therapy for PKU.

Tissue Specific Enhancers

H 018 CHARACTERIZATION OF THE CELLULAR TRANSCRIPTION COMPLEX AT THE HIV PROMOTER, Katherine A. Jones, Harold Dinter, Nathalie Duchange, Marina Schorpp, The Salk Institute Molecular Biology and Virology Laboratory, San Diego, California 92138

The relatively compact promoter regions of the human immunodeficiency viruses, HIV-1 and HIV-2, are strongly modulated by various agents that activate protein kinase C (e.g., the phorbol ester tumor promoter, TPA) and by viral proteins (tat-1 and tat-2) that act through unique sequences downstream of the RNA start site. To obtain a more complete understanding of these processes, we have investigated the function of these promoters and selected mutants with in vitro transcription and DNA-binding experiments. The HIV enhancer is recognized by a DNA-binding protein active in TPA-treated HeLa cells and in uninduced B-lymphoid cells. Extracts prepared from TPA-induced or mock-induced HeLa tk- cells recapitulated the induction by TPA of the DNA-binding activity of this protein and demonstrated a concomitant increase in transcriptional activity that was dependent on intact enchancer sequences. A protein with similar DNA recognition properties has been purified from a constitutive B cell source by a combination of conventional and sequence-specific affinity chromatography, and an analysis of its relative transcriptional activity and potential relatedness to the HeLa factor is in progress. Two distinct affinity-purified factors recognize adjacent sites within the promoter-proximal domains of both HIV-1 and HIV-2. We have analyzed the interaction the interaction of these proteins with various linker-scanning mutants in the HIV-1 leader (provided by P.A. Luciw, U.C. Davis) to determine individual nucleotide contacts and the extent to which these proteins may be involved in establishing the basal transcriptional efficiency or response to viral regulation. The role of these proteins in cellular transcription events is also under investigation.

Gene Transfer and Gene Therapy

Recombination

H 01? SITE-DIRECTED MUTAGENESIS BY GENE TARGETING IN MOUSE EMBRYO-DERIVED STEM CELLS, Mario R. Capecchi and Kirk R. Thomas, Department of Biology, University of Utah, Salt Lake City, Utah 84112.

We mutated, by gene targeting, the endogenous hypoxanthine phosphoribosyl transferase (HPRT) gene in mouse embryo-derived stem (ES) cells. A specialized construct of the neomycin resistance (neo') gene was introduced into an exon of a cloned fragment of the Hprt gene and used to transfect ES cells. Among the G418^r colonies, 1/1000 were also resistant to the base analog 6-thioguanine (6-TG). The G418^r, 6-TG^r cells were all shown to be Hprt$^-$ as the result of homologous recombination with the exogenous, neo'-containing, Hprt sequences. We have compared the gene-targeting efficiencies of two classes of neo'-Hprt recombinant vectors: those that replace the endogenous sequence with the exogenous sequence and those that insert the exogenous sequence into the endogenous sequence. The targeting efficiencies of both classes of vectors are strongly dependent upon the extent of homology between exogenous and endogenous sequences. The protocol described herein should be useful for targeting mutations into any gene.

H 020 EARLY AND LATE STEPS IN HOMOLOGOUS RECOMBINATION IN CULTURED MAMMALIAN CELLS
R. Michael Liskay and Alan S. Waldman, Depts. of Therapeutic Radiology and Human Genetics, Yale School of Medicine, New Haven, CT 06510

Conceptually, the pathway of homologous, or general genetic recombination can be divided into three phases; 1) initiation, which is usually equated with a search for homology and the initial pairing of strands 2) middle events, strand exchange and formation of a "Holliday structure" followed by branch migration or movement of the "Holliday structure" and 3) resolution, cleavage of the "Holliday structure" to yield the final products. Our lab has employed defined selectable markers (tk genes) as substrates integrated as closely-linked repeats in the genome of mammalian cells in order to conduct a molecular genetic analysis of both "early" and "middle" events in recombination. In one such investigation we have found that small amounts of heterology between two genes if distributed appropriately, e.g. two single base pair mismatches in an otherwise perfect stretch of 230bp, can reduce the rate of recombination by 20X relative to the homologous control "cross". In a second heterologous cross there are four mismatches present that do not interrupt a "long" stretch of perfect homology and the rate of recombination is unaffected relative to the homologous cross. Taken together these results indicate that a crucial factor in determining rates of intrachromosomal recombination is the length of uninterrupted homology rather than the overall homology. Further studies suggest that small amounts of heterology affect both early and later steps in recombination. Therefore a relatively large minimum target size is required for efficient chromosomal recombination in mammalian cells. In E. coli, the corresponding target size is only 25-50bp. Relevant to the end of the pathway, we have identified and are in the process of purifying an activity from human cells which carries out cleavage of substrates designed to mimic "Holliday structure" intermediates. This activity cleaves the structure in a manner expected for a "resolvase" enzyme. Progress on the purification and characterization of this activity will be presented.

Late Addition

H 021 MOLECULAR GENETICS OF STEROID AND THYROID HORMONE RECEPTORS, Ronald M. Evans,
Howard Hughes Medical Institute, The Salk Institute, La Jolla, CA 92037.
Steroid receptors are members of a superfamily of regulatory genes whose analyses are important for understanding molecular details of transcriptional control as well as providing insight into how individual trans-acting factors contribute to cell identity and function. Mutational studies reveal these receptors to be composed of discrete functional domains. By interchanging these domains, it is possible to create novel, functional, hybrid receptors. This family encodes receptors for remarkably diverse ligands including steroid and thyroid hormones, and the vitamin A-related morphogen retinoic acid. Although animals develop in various and complex ways, the discovery of receptor-related molecules in a wide range of species, suggests that molecular mechanisms underlying development and physiologic homeostasis may be more common than was previously suspected.

Gene Transfer and Gene Therapy

Vectors and Gene Transfer

H 100 HIGH LEVEL REGULATED EXPRESSION OF THE HUMAN BETA GLOBIN GENE AFTER RETROVIRAL TRANSFER INTO MEL CELLS AND HUMAN BFU-E

M. Bender, A.D. Miller and R.E. Gelinas. Department of Pathology, University of Washington, and the Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Retroviruses capable of transducing the human β globin gene were constructed in order to study regulation of the transduced β globin gene in transformed and normal hematopoietic cells, and to investigate their potential for somatic gene therapy. A six base pair insertion was placed in the 5' untranslated region of a β gene to allow transcripts from the transduced and the endogenous globin genes to be distinguished. This mutation did not alter transcript initiation or accumulation. Replication-defective amphotropic retrovirus vectors containing the neomycin resistance gene and either the marked β globin gene with introns or an intronless minigene were constructed and used to study β globin expression in MEL cells and BFU-E's cultured from human bone marrow.

Viral transfer of the β globin gene into MEL cells resulted in inducible expression of human β globin RNA. The transduced β globin gene was expressed at levels similar to the endogenous mouse β globin genes. Human β globin peptide was detected by immunofluorescence in induced cells, but not in uninduced cells. In contrast, no expression was detected from the virally transduced β globin minigenes, suggesting that β globin introns are necessary for expression. Evaluation of β globin genes containing either the first intron or the second intron reveal that the second intron contains a cis-acting determinant which increases RNA accumulation.

Virus containing the marked β globin gene was used to infect human bone marrow and BFU-E's were cultured. Expression of the virally transduced β globin gene was detected in G418 resistant BFU-E's.

H 101 A NOVEL INDUCIBLE TOXIC VECTOR SYSTEM FOR MAMMALIAN CELLS, Emiliana Borrelli, Richard Heyman, Mary Hsi, and Ronald M. Evans, Salk Institute, La Jolla, CA 92037

Gene transfer technology has provided a valuable approach to impart new phenotypic properties on cultured cells. Certain vectors expressing bacterial enzymes that confer resistance to cytotoxic drugs such as neomycin have been useful in providing a universal dominant selectable marker for sensitive selection of co-transfected plasmids in virtually any cell line. A potentially valuable adjunct approach would be to introduce negative selectable markers into cells that would either inhibit cell proliferation or lead to cellular degeneration. To explore these possibilities is first necessary to establish an experimental approach, both the for the expression of a potentially toxic product and its manipulation in vitro. Here we describe the strategy to the generation of an inducible toxic vector system that will have specific application in the analysis of cell lineage formation. This strategy is based on the selective expression of herpes thymidine kinase (HSV1-TK) which is able to metabolize the synthetic nucleoside analogs ACV and FIAU, to toxic nucleotide precursors. Incorporation of the phosphorylated form of these drugs by the cellular DNA polymerase leads to chain termination of replicating DNA and subsequently cell death. This has been tested either when the HSV1-TK gene is present in an episomal form in the cell or when it is stably integrated in a cell line. We speculate that should be possible to take advantage of these properties to create a novel toxic vector system in transgenic animals. Such a system will take advantage of the ability of tissue specific promoters to target restricted expression of this function in developing cells.

H 102 RETROVIRAL GENE TRANSFER OF BETA-NERVE GROWTH FACTOR INTO CULTURED CELLS, Xandra O. Breakefield[1], M. Priscilla Short[1], David Wolf[2], Michael B. Rosenberg[3], Christiana Richter-Landsberg[1], Constance Cepko[2], Theodore Friedmann[3] and Fred H. Gage[3], [1]E.K. Shriver Ctr., Waltham, MA 02114; [2]Harvard Med. Sch., Boston, MA 02115 [3]Univ. Calif. Sch. Med., La Jolla, CA 92093.

Retroviral vectors have been constructed containing cDNAs equivalent to different forms of the mRNA for mouse beta-nerve growth factor (NGF) under the control of the LTR promoter, as well as the gene conferring resistance to G418 under the control of the SV40 early promoter. Vectors have been used to confer expression of biologically active NGF onto several cell types in culture, including a mouse pituitary line and primary astrocytes. Virally encoded NGF has been evaluated by Northern blot analysis, immune precipitation and SDS-PAGE, and two-site radioimmunoassay. Cells transduced in culture have been transplanted into adult rat brains to evaluate their survival and ability to supply NGF to responsive, cholinergic neurons.

H 103 A METHOD TO DETECT TRANSFECTED CHLORAMPHENICOL ACETYL TRANSFERASE GENE EXPRESSION IN INTACT BONE MARROW RECONSTITUTED MICE.
C.F. Chiu,+ M.M. Jastreboff,* E. Ito,+ J.R. Bertino+ and R. Narayanan+. +Memorial Sloan-Kettering Cancer Center, New York, NY 10021, *Dept. of Surgery, yale Univ. Sch. of Med., New Haven, CT 06510.

A rapid procedure is developed for assaying chloramphenicol acetyl transferase (CAT, EC 23.1.28) enzyme activity in intact mice following transfection of the RSV CAT plasmid into mouse bone marrow cells by electroporation. The reconstituted mice were injected with [C^{14}] chloramphenicol by tail vein and ethyl acetate extracts of 24h urine samples were analyzed by TLC autoradiography for the excretion of labeled metabolites. CAT expression in vivo was detected by the presence of labeled acetylated metabolites in the urine within one week after bone marrow transplantation and, under the conditions described, these metabolites were detected for at least three months. The urine of control mice did not show the presence of labeled metabolites following [C^{14}] chloramphenicol injection. CAT expression in intact mice as monitored by the urine assay correlated with CAT expression in the hematopoietic tissues assayed in vitro. This method offers a rapid method of screening for introduced CAT gene expression in vivo without the need to sacrifice the mice.

H 104 Fate of murine helper virus in non-human primates.
Ken Cornetta, Robert Moen, Al Gillio, Ken Culver, Robert Wieder, R. Michael Blaese, Richard O'Reilly and W. French Anderson. Laboratory of Molecular Hematology, NHLBI/NIH, Metabolism Branch, NCI/NIH, Bethesda Md. and Memorial Sloan Kettering Cancer Center, N.Y., N.Y.
Human gene therapy may inadvertently expose patients to replication competent murine retroviruses. The following studies were performed to assess the risk factors and safety of retroviral mediated gene transfer in humans. (A) Three rhesus monkeys received IV infusion of 50 ml of S3A supernate (Moloney based pSAX vector packaged in PA12, titer $5x10^6$ and 0.1% helper virus) mixed to contain 0, 70 or 85% supernate from cells producing the amphotropic helper virus 4070A (titer >10^4). Serum analysis using the S+/L-assay revealed rapid clearing of retrovirus (within 15-30 min) and subsequent evaluations have been negative, up to 4 months after injection. The animals have been asymptomatic throughout the study. Seven days after injection, two animals developed lymphadenopathy that resolved within 10 days. S+/L-, NPT and human ADA analysis of the lymph node tissue and cultured lymphocytes have been negative. (B) Additional information was obtained by surveying primates which have undergone gene transfer experiments involving bone marrow transplantation. All animals tested are S+/L- negative, up to 2 years after exposure to helper contaminated retroviral vectors. Our data suggests that amphotropic Moloney based helper virus may not be capable of establishing a persistent viremia in primates.

H 105 RETROVIRAL-MEDIATED GENE TRANSFER INTO CULTURED LYMPHOID CELLS AS A VEHICLE FOR GENE THERAPY.
Ken Culver, Scott Freeman, Don Kohn, Michael Wood, W. French Anderson, Jay A. Berzofsky, and R. Michael Blaese. NCI and NHLBI, National Institutes of Health, Bethesda, MD 20892
Most experimental systems for gene therapy have focused on bone marrow as the tissue of choice for gene insertion because it is a self-renewing pluripotent progenitor cell population, potentially allowing delivery of genes into erythroid, myeloid and lymphoid lineages. Low level expression in peripheral cells has been common, perhaps because multiple steps of differentiation may lead to inactivation of the inserted gene. As an alternative approach, we are inserting various genes into differentiated lymphocyte populations in vitro and then reintroducing the cells. Antigen specific T-cell lines and clones from mice, rats, rhesus monkeys and humans have been established in culture by repeated cycles of stimulation and resting on syngeneic or autologous lymphoid feeder cells. Retroviral vector constructs containing genes for human ADA (SAX) or rat growth hormone (PG2N10) and neomycin resistance (neoR) as a dominant selectable marker were prepared. Murine T-cell lines specific for sperm whale myoglobin were infected with SAX or PG2N10 after proliferation was induced by antigen. T-cell colony formation in methylcellulose +/- G418 demonstrated an efficiency of infection of > 50% for SAX. G418 selected SAX infected mouse cells produced human ADA at levels equivalent to endogenous ADA. The supernatants from the PG2N10 infected T-cells contained significant rat GH. Rat, monkey and human T-cell lines also expressed NeoR after infection with these vectors. In addition, lymphocyte lines established from an ADA(-) SCID patient produced normal amounts of ADA and detoxified deoxyadenosine and Xyl-A after infection with the SAX vector. These results show that T-lymphocyte lines can be readily infected with retroviral vectors and express a variety of genes stabily over many generations. These antigen specific T-cells are responsive to defined antigens and thus have the potential to be expanded by antigenic challenge in vivo after reintroduction into an intact animal. Experiments evaluating the duration of survival of such reintroduced T-cells as well as their expression of hADA, rGH and NeoR in nude, SCID, hypophysectomized and intact mice will be presented.

Gene Transfer and Gene Therapy

H 106 **Expression of retroviral trans-acting functions from complementary crippled genomes: a system for helper free packaging of retroviral vectors.** Olivier Danos and Richard Mulligan, Whitehead Institute for Biomedical Research, 9 Cambridge center, Cambridge, Ma. 02144.

Packaging cell lines for retroviral vectors which have been used hitherto, such as Ψ2 or Ψam were constructed by transfecting into NIH3T3, cloned Mo-MuLV helper genomes rendered defective for packaging by a cis-acting 350 bp deletion (Ψ^-). However, this deletion has proven to be leaky and Ψ^- genomes can be packaged and transferred to an infected cell at a low frequency (1 in 10^3). If several round of infection are performed on the same cell population or if cocultivation is used, it becomes likely that "secondary producers" will arise, that is cells which will be doubly infected by a recombinant virus and by the Ψ^- virus. This can seriously complicate, if not prevent, any kind of lineage analysis. A second problem may arise, since the Ψ^- genome and a recombinant genome carrying the Ψ sequences can be copackaged in heterozygous virions. Due to the property of the viral polymerase to frequently switch templates, a replication competent helper virus can easily be formed during reverse transcription of such an heterozygous particle. Therefore, in order to eliminate the transfer of the packaging functions as well as the possibility of helper virus formation, we have constructed trans-acting genomes which, in addition to the Ψ^- deletion, contain the following features: 1) alterations have been introduced in the cis acting sequences required for reverse transcription and integration; 2) since such alterations can always be rescued upon recombination with a copackaged recombinant construct, the genomes also bear non rescueable frameshift mutations in the trans acting functions. We have constructed two sets of complementary crippled genomes(one ecotropic and one amphotropic) carrying mutations either in the $gp70^{env}$ coding sequences or in the gag-pol gene. These molecules were introduced in NIH 3T3 cells by two successive rounds of transfection/selection. We have derived two packaging cell lines producing virions with amphotropic (Ψ CRIP) or ecotropic (Ψ CRE) specificities. Producer lines isolated in our laboratory with more than 15 different constructs have proven to be stable, produce virus stocks with high titers (10^5 to 10^6 infectious particles/ml), do not transfer the packaging functions and are free of helper virus. Cocultivation of explanted mouse bone marrow with some of these producers resulted in infection of hematopoietic stem cells. Ψ CRIP is potentially a suitable and safe tool for the production of recombinant retroviruses to be used in somatic gene transfer to large organisms.

H 107 EXPRESSION OF NEW ANTIGEN-SPECIFICITY IN T CELLS AFTER TRANSFER OF TCR GENES BY RETROVIRAL VECTORS. G. De Libero, L.Mori, M.Righi, N.Malgaretti°, R.Mantovani°, P. Comi°, S. Ottolenghi#, & P. Ricciardi-Castagnoli, CNR Ctr. Cytopharmacology, °Ctr. Study of Cell. Pathology, #Dept. of Genetics, Univ. of Milano, Milano, Italy.

Retroviral vectors containing the T-cell antigen receptor α and β genes have been constructed. The cDNA sequences of these genes, derived from a murine cytotoxic T-lymphocyte, have been inserted in the pLJ plasmid containing LTR and ψ regions of the MoMuLV, SV40 promoter and neomycin resistance gene. Three different constructs have been used to transfect ψ_2 cells and G418-resistant clones analyzed by Southern blotting in order to identify the ψ_2 clones containing the complete proviral sequences. The ψ_2 clones showing the highest titers of viral particle production have been selected. These viruses have been used to infect different mouse cell lines. The expression of the T-cell receptor genes in various cell populations has been analyzed using specific probes. The appearance in the cytoplasm or in the cellular membrane of the α and β proteins have been studied using monoclonal antibodies.

Cytotoxic T lymphocytes, after infection, express the new TCR and acquire the new antigen-specificity. Using the limiting dilution culture system and ^{51}Cr release assay, the frequency of retrovirus-infected cytolytic T cell precursors with the new antigen-specificity was increased four fold with respect to uninfected cells. Helper T lymphocytes were also infected and found able to express the new TCR on the surface membrane. However, these cells were not functional when assayed for IL-2 production after stimulation with the new antigen.

H 108 GENETIC MANIPULATION OF HUMAN HEMATOPOIETIC CELLS WITH RETROVIRUS VECTORS. John E. Dick*, Wayne Chang*, Pierre Laneuville#, Axel A. Fauser#, Dept. of Genetics*, Research Institute, Hospital for Sick Children, and Dept. of Medical Genetics, University of Toronto, Toronto, Ontario; Division of Haematology and Ludwig Institute, Royal Victoria Hospital, McGill University, 687 Pine Ave. West, Montreal, Quebec, Canada.

The ability to transfer new genetic information into human hematopoietic stem cells offers a novel approach for understanding the genetic events governing stem cell development. Moreover, introduction of cloned genes into human hematopoietic cells opens up the possibility of correcting certain human diseases by gene therapy. We have recently carried out experiments aimed at increasing the efficiency of retrovirus mediated gene transfer into human progenitor cells. By applying several modifications to existing protocols, including the addition of hematopoietic growth factors and preselection, high efficiency gene transfer into normal human bone marrow cells has been obtained. Using in vitro colony assays, the proportion of progenitor cells expressing the neo gene was between 70 to 100%. A variety of vectors using the human actin, HSV-TK, or LTR promoter were also introduced and expressed in human bone marrow progenitor indicating these vectors are active in human hematopoietic cells at least for 14 days during colony formation in vitro.

H 109 EXPRESSION OF HUMAN α-GLOBIN GENE IN MOUSE BONE MARROW CELLS, Varavani J. Dwarki, Chung-leung Li, Pamela Mellon and Inder M. Verma, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Human α-globin gene was introduced into N2 based retroviral vector in an orientation opposite to that of viral 5' LTR. Recombinant viruses with titers ranging between 5×10^5 - 8×10^5 CFU/ml (Neo resistant) were obtained from helper independent Ψ2 cell line. The 1.5 kb human α-globin gene contained 570 bp of 5' flanking sequences and 90 bp of 3' flanking sequences in addition to the three exons and two introns. Analysis of DNA and RNA from the Ψ2 cell lines revealed the expected size fragments and transcripts. Furthermore, the α-globin mRNA has accurate initiation as judged by SP6 protection assays.

Bone marrow from normal mice was co-cultivated with Ψ2 cells producing α-globin recombinant virus. G 418 resistant bone marrow cells were injected into irradiated mice and 12 day spleen colonies were assayed for both proviral integration and expression of the globin mRNA. Over 90-95% of the spleen colonies showed integration of the proviral copy and most of the spleen colonies also showed expression of globin mRNA. Quantification of human α-globin mRNA and mouse endogenous α-globin mRNA was carried out by SP6 protection assay. It appears that the exogenously inserted human α-globin gene is expressed at levels which are about 20-fold lower than the endogenous gene. We are currently in the process of identifying the α-globin protein by immunofluorescence using specific antibodies.

Supported by NCI.

H 110 LINEAGE-SPECIFIC EXPRESSION OF A RETROVIRALLY TRANSDUCED HUMAN β-GLOBIN GENE IN MURINE BONE MARROW TRANSPLANT RECIPIENTS, Elaine A. Dzierzak and Richard C. Mulligan, Whitehead Institute, Cambridge, MA 02142.

Retroviruses have been used to efficiently introduce exogenous genes into bone marrow stem cells for the permanent engraftment of lethally irradiated adult mice. Although proviral sequences have been demonstrated to be present in all hematopoietic lineages of such mice long after transplant, expression of the introduced genes has been problematic. While most studies have utilized retroviral transcriptional sequences for expression of inserted cDNAs, it may be important to retain cellular transcriptional sequences normally active in hematopoietic cells for efficient expression of transduced genes in the bone marrow transplant system. Therefore we introduced recombinant retroviruses containing genomic human β-globin coding and regulatory sequences into murine hematopoietic stem cells. Four to nine months after transplant of male recipients with infected female bone marrow, we observed intact proviral sequences in spleen and bone marrow DNA at copy numbers of 0.02 to 0.4, with the efficiency of infection in individual animals ranging from 2% to 100%. When peripheral blood RNA from eight mice was examined by SP6 protection analysis for human β-globin transcripts, the level of human β-globin mRNA varied between 0.4% to 4.0% of endogenous mouse β-globin mRNA (at a single copy per genome). Expression of human β-globin protein was also observed when erythrocytes from these animals were stained with human β-chain specific monoclonal antibody, with the percentage of immunofluorescent cells approximating the proviral copy number as determined by Southern blot analysis. Expression of human β-globin is erythroid-lineage specific as demonstrated by SP6 protection analysis of fractionated hematopoietic cells in these animals. Thus, genomic sequences can function in a normal, regulated manner in the context of retroviral sequences after stem cell infection and (with inclusion of additional sequences known to increase human β-globin expression to endogenous levels) can be potentially useful for somatic cell gene therapy of human disorders such as β-thalassemia and sickle cell anemia.

H 111 EFFECT OF COLONY STIMULATING FACTORS ON THE INFECTION OF HUMAN BONE MARROW WITH RETROVIRAL VECTORS, Martin A. Eglitis and W. French Anderson, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892.

Retroviruses have been used as vectors to introduce exogenous genes into human bone marrow cells *in vitro*. For the genome of the vector to be integrated within the genome of the target cell, it is postulated that the DNA of the cell must undergo a round of replication. However, the target stem cells and their daughter progenitors are relatively quiescent which may account for the observed infection efficiencies of 1-10%. We have undertaken an initial evaluation of the potential of GM-CSF and IL-1a to stimulate BFU-E and CFU-GM, thereby improving the efficiency with which genes are introduced into them with retroviral vectors. Nucleated bone marrow cells were obtained from normal volunteers and were either immediately infected by cocultivation with PA317 packaging cells producing N2 vector, or first cultured separately for 48 hr before infection. Depending on the particular experiment, culture medium was supplemented with 10% PHA-stimulated lymphocyte-conditioned medium (PHA-LCM), IL-1a, or GM-CSF either individually or in combination. When marrow was directly infected with virus, so long as any growth factors were present, 1-2% of recovered colonies were infected with N2. After prestimulation in either PHA-LCM or GM-CSF, infection efficiencies were improved up to 10 fold. Analysis of cell cycling in the treated marrow by thymidine uptake confirmed that some growth stimulation was obtained by treatment with growth factors. These results indicate that by using colony stimulating factors to induce cell division in marrow cells, some improvement in the efficiency of gene transfer can be obtained.

H 112 DETECTION BY FLOW CYTOMETRY OF VIABLE MOUSE HEMATOPOIETIC CELLS EXPRESSING RETROVIRALLY TRANSDUCED ß-GALACTOSIDASE GENES, Paul W. Eldridge and Gary Van Zant, Department of Cell Biology and Anatomy, Texas Tech Health Sciences Center, Texas Tech University, Lubbock, TX 79430

We have developed a method whereby the expression of _E. coli_ ß-galactosidase (ß-gal), transduced into mouse hematopoietic stem cells with the Ψ-2 BAG1 retrovirus vector, can be detected in viable cells with a flow cytometer and a fluorometric assay for ß-gal activity. The assay involves incubation of the cells with the synthetic substrate, fluorescein-ß-d-galactopyranoside. Cleavage of this substrate by ß-gal releases intracellular fluorescein, which can be detected with the flow cytometer.

Levels of ß-gal activity were determined in blood cells of radiation chimeras. Increased enzyme activity was detected in cells sets of mice infected with the experimental vector containing the ß-gal gene when compared with cells of mice infected with a control vector lacking the ß-gal gene. Southern blot analysis confirms the presence of the foreign insert in mice with increased ß-gal activity. We noted some variability in the relative activity among the positive animals. This may reflect differences from animal to animal in the utilization of infected stem cell clones.

Previous use of ß-gal as a marker for the expression of foreign genes has usually been accomplished with histochemical stains or other retrospective techniques. The novel use of the flow cytometer to detect intracellular expression of a transgene in viable cells should prove to be a very useful tool. Cells can be quickly assayed and sorted for use in subsequent experiments. The inclusion of ß-gal in vectors with other genes of interest should provide a marker for the expression of genes that are less easily detected.

H 113 EXPRESSION OF THE HUMAN α-GLOBIN GENE IN RETROVIRALLY TRANSDUCED HAEMATOPOIETIC CELLS _IN VIVO_ Mitchell Finer and Richard Mulligan, Whitehead Institute, Cambridge MA. 02142

Many laboratories have demonstrated that retroviral vectors can be used to introduce foreign genes into bone marrow cells _in vivo_ with high efficiency.Previous work has focused on the expression of cDNAs in direct orientation either from LTR based vectors or from a variety of internal promoters. Although high efficiency of gene transfer into bone marrow in long term reconstituted animals has been reported, these constructs express poorly or not at all _in vivo_. Instead, we have examined the expression of a 1.5 kb Pst I fragment encoding the complete human α1-globin gene and flanking sequences, cloned in reverse orientation into the retroviral vector pSG. pSG contains the 5' LTR and the first 1.4 kb of the gag region up to the Xho I site of Mo-MuLV joined by a Xho linker to the final 150 bp of Mo-MuLV from the Cla I site and the 3' LTR. The 3' LTR contained the Pvu II-Xba I enhancer deletion. This approach alleviates the artificial juxtapositioning of different promoter elements with cDNA coding sequences, during which sequences essential for mRNA stability, transport or correct translation might be improperly placed or deleted. Three ψ-2 producer clones have been isolated which transmit the correct viral structure at 20-30 copies per genome, assayed by 3T3 infection and southern blotting. These producers were used to infect mouse bone marrow in vitro followed by engraftment of lethally irradiated recipients .The efficiency of short and long term engraftment with retrovirally marked stem cells was assayed by southern blotting. In addition, the expression of the transduced α-globin gene was assayed by SP6 mRNA protection. Seventy-five percent of the 14-day spleen colonies isolated were positive for single copy provirus integration at a unique site. Of these, 33% were positive for expression of human α-globin mRNA. The level of expression was position dependant. The highest levels of expression were comparable to that of the endogenous mouse γ-actin gene. Three months following transplant, DNA and RNA was isolated from blood, bone marrow, spleen and spleen B cells. Three animals were positive for integration in spleen and bone marrow DNA, the best animal containing a single copy of the provirus. SP6 mRNA protection revealed poor expression in blood, bone marrow and spleen B RNA, but high expression in total spleen RNA. This work demonstrates that the human α1-globin gene can be efficiently introduced into long term reconstituting cells. However, its expression is not specific to the erythroid lineage.

H 114 MURINE RETROVIRUS PARTICLE TITER AND INFECTIVITY USING DIFFERENT HOST CELL PACKAGING LINES, Scott Freeman, Daryl D. Muenchau, James A. Zwiebel, and W. French Anderson, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892.

Retroviral packaging cell lines have been generated using murine fibroblasts. These lines produce viral titers up to 10^7 cfu/ml. During the process of viral particle production, virions bud through the cell membrane and carry with them membrane proteins from the host cell. We undertook a study to determine if a packaging line derived from a different tissue and species would 1)enable higher viral particle production, and 2) produce a relatively higher titer on cells of a common tissue or species. A human hepatoma cell line (HepG2) was cotransfected with pN2 (a neo^R vector) and pPAM3 (a plasmid that provides _trans_ functions and possesses the 4070A amphotropic envelope gene). Twenty four clones were isolated and screened for viral titer. The highest titer clone, designated L1, produced 10^4 cfu/ml using NIH-3T3 cells. We then compared the L1 packaging line to two other packaging cell lines established in mouse and mink fibroblasts by transfecting pN2 and pPAM and expressing viral titers of 10^6 and 10^2 respectively.Supernatants from the three packaging cell lines were then titered onto seven different cell lines: human fibrosarcoma, embryonal rhabdomyosarcoma, hepatoma, and SV40-transformed lung fibroblast cells; mouse 3T3 cells; and mink fibroblasts. Viral particle stability as measured by resistance to freeze-thaw and incubation at 37 C was similar among the different packaging lines. Viral supernatants of a given titer gave proportionately similar titers on each target cell type. The presence of similar membrane components on the viral envelope and the target cell did not affect viral infectivity.

H 115 PSEUDOVIRION–MEDIATED GENE TRANSFER. Susan E. Gould–Fogerite, James J. Reston, Raphael J. Mannino. Albany Medical College, Albany NY 12208.

Pseudovirions, proteoliposomes containing the envelope glycoproteins of Sendai or influenza virus integrated in the lipid bilayer and DNA encapsulated within the aqueous interior, can mediate gene transfer to animals and to cells in culture. Sendai pseudovirions prepared by the protein cochleate method (Anal. Biochem. 145:15–28 [1985]) have been used to stably transform mouse C127 cells with a bovine papilloma virus–based plasmid at 100,000 times greater efficiency than calcium phosphate precipitation, with respect to the quantity of DNA needed to achieve maximal transfection. Preliminary data indicates stable transformation of primary hepatocytes at efficiencies similar to retro–viral mediated gene transfer.

Influenza pseudovirion mediated gene transfer to cells in culture achieved transient expression of DNA in 100% of cells with maximal expression occurring 6–12 hours after low pH activated fusion of cell–bound vesicles.

Pseudovirions containing a plasmid encoding polyoma virus early proteins were injected subcutaneously in the suprascapular region of newborn mice. Tumors which arose 6 to 7 months later were shown to contain the transferred DNA and express the polyoma pro–teins if encoded. Gene transfer to 50% of the mice in one experimental group was achieved.

H 116 DEVELOPMENT OF RETROVIRAL VECTORS USEFUL FOR EXPRESSING GENES IN CULTURED MURINE EMBRYONAL CELLS AND HEMATOPOIETIC CELLS IN VIVO, Braydon C. Guild and Richard C. Mulligan, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA, 02142.

In order to generate retrovirus vectors that would be generally useful for gene expression studies in a variety of developmental contexts, we have constructed and tested a series of vectors that employ cellular transcriptional signals, derived from the chicken β-actin, human histone H4, or mouse thy-1 genes, known to be functional in either early embryonal cells or in cells representing a variety of stages of hematopoietic cell differentiation. One of the vectors, HSG-neo, which contains the human histone H4 promoter, was shown to yield reasonable levels of RNA in hematopoietic cells derived from long term reconstituted bone marrow transplant recipients engrafted with transduced stem cells. The HSG-neo vector yields extremely high titer virus producing cell lines, and is transcriptionally active in both F9 cells and hematopoietic cells in vivo. Expression studies with a large number of different inserts including the genes for human adenosine deaminase and murine multidrug resistance are now in progress.

H 117 TRANSFER OF CHICKEN MHC GENES INTO THYMIC EPITHELIUM, François Guillemot, Charles Auffray and Nicole M. Le Douarin, Institut d'Embryologie du CNRS et du Collège de France, 49bis, Avenue de la Belle-Gabrielle 94736 NOGENT-sur-MARNE CEDEX France.

We have developed a model of immune tolerance induction during embryonic life. Xeno- or allografts of limbs or neural tubes, made between avian embryos, are rejected after hatching unless an epithelial thymic primordium is grafted at the same time. Since Major Histocompatibility Complex (MHC) molecules are expressed by thymic epithelium and could be involved in the process of tolerance induction, we have cloned MHC class I and class II genes in the chicken species. We are now using retroviral vectors to transfer MHC genes into thymic epithelium and study how the products of these transgenes, when expressed in the thymus, can participate to the tolerization to foreign tissues during embryonic development.

H 118 SPECIES AND CELL TYPE DEPENDENT EXPRESSION EFFICIENCY AND BPV VECTOR STABILITY, Lennart Hansson, Eva Lagerholm, Björn Löwenadler, Gunnar Pohl, Kerstin Schenström and Catharina Sterky, KabiGen AB, S-112 87 Stockholm, Sweden

Bovine papilloma virus expression vectors carrying the human tissue plasminogen activator (t-PA) cDNA as a reporter gene were co-transfected with a vector encoding neomycin resistance.
Clones were selected by growth in medium supplemented with G418.
Two vectors with different upstream transcription regulatory elements were transfected into a set of cell lines derived from various mammalian species. The relative expression efficiency in the various cell types was monitored by determining extracellular t-PA antigen levels.
Another aspect of this study was to analyse the capability of the cell lines to support stable episomal replication of BPV vectors.
The results demonstrate that SV40 early enhancer/promoter element directs considerable expression of t-PA in hamster cells but is less active in mouse cells in contrast to the mouse MT-1 element which is more effective in mouse cells.

H 119 THE INTRODUCTION OF CHIMERIC GENES CONTAINING THE PEPCK PROMOTER-REGULATORY REGION CELLS AND ANIMALS USING RETROVIRUSES M. Hatzoglou, W. Lamers, A. Wynshaw-Boris, H.C. Haung and R.W. Hanson, Department of Biochemistry, Case Western Reserve University, Cleveland OH 44106.

A series of chimeric genes containing a 547 bp segment of the P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) promoter-regulatory region ligated to the structural genes for neo or bovine growth hormone (bGH) were introduced into the murine retroviral vector, pLJ (from R. Mulligan, M.I.T.). Infectious virus derived from this vector via $\psi 2$ cells, were used to infect hepatoma cell lines and fetal rats in utero. Southern blot analysis of DNA isolated from both the cells and animals indicated a single site of integration of the provirus, dependent on the presence of PEPCK promoter sequences in the retroviral vector. Transcription of the PEPCK-neo gene in infected FTO-2B hepatoma cells was stimulated by the addition of glucocorticoids and cAMP; this stimulation was blocked by the simultaneous addition of insulin. mRNA transcribed from the PEPCK-neo gene was detected in the livers of adult rats, which had been injected in utero at 18-20 days of development. The expression of this chimeric gene was generally stimulated by the administration of cAMP or thyroxine to the animals after birth. When two chimeric genes, PEPCK-neo and PEPCK-bGH were ligated in opposite orientation between the LTR's of the retrovirus and infected into FTO-2B cells, the rate of gene transcription from both promoter was regulated by hormones in a manner similar to that noted when a single chimeric gene was contained in the virus. These studies suggest that retroviral vectors containing the PEPCK promoter are potentially useful for introducing regulated genes into cells and animals.

H 120 **GENE TRANSFER INTO HEMATOPOIETIC STEM CELLS MEDIATED BY AN ADENO-ASSOCIATED VIRUS VECTOR,** Paul L. Hermonat*, Drake M. LaFace, Edward K. Wakeland, and Ammon B. Peck, Dept. of Pathology, Univ. of Florida, Gainesville, FL 32610, *present address: LTVB, NCI, Bethesda, MD 20892. Studies have shown that adeno-associated virus (AAV) is a high efficiency transducing vector in tissue culture. An AAV/Neomycin^R recombinant virus stock can transform 10% of human D6 cells to G418 resistance at a high multiplicity of infection (moi) and the host range of AAV provirus integration appears to be broad. We used this same recombinant virus to transduce primary murine hematopoietic progenitor cells to G418 resistance. In three experiments we infected the freshly explanted bone marrow cells (B10.BR/cd mice) with the AAV/Neo^R recominant virus stock at an moi of 1 or 10 for two hours before culture in soft agar. Selection, 1.2 mg/ml G418, was applied at 24 hours and colony numbers determined at one week. G418 resistant colonies were obtained at about 0.5 to 1.5% efficiency compared to cells infected by the vector but not selected. The resistant colonies were normal and compact containing several hundred members and appeared to be of mixed GM or GEM type. No naturally resistant cells were found to the G418 levels used for selection in these experiments or any other colony forming assay we performed. Thus, these data suggest that AAV based vector systems are useful for the transformation of bone marrow stem cells and can be used as an alternative to the retrovirus based vector systems used for the transduction of primary cells.

Gene Transfer and Gene Therapy

H 121 RELATIVE EXPRESSION LEVELS OF CAT IN AVIAN FIBROBLASTS UNDER TRANSIENT AND STABLE CONDITIONS, Paul Hippenmeyer, Monsanto Company, St. Louis, Mo 63198.
Avian retrovirus vectors were constructed to evaluate the relative efficiency of promoters placed internal to the viral LTRs. The vectors are replication-defective reticuloendotheliosis plasmids that contain the neomycin phosphotransferase (NPT-II) gene under control of the 5' LTR and an internal promoter that directs expression of the chloramphenicol acetyltransferase (CAT) gene. The internal promoters are the SV40 early, the mouse metallothionein I (mMT-1) and the human cytomegalovirus (CMV) immediate early promoters. Under transient conditions in QT6 cells, the CMV promoter construct produced 63- and 26-fold higher amounts of CAT than the mMT-1 and the SV40 promoters, respectively. QT6 cell lines harboring each construct were constructed after transfection of the vector DNA and selection for G418 resistance. Pooled colonies were assayed for NPT and CAT activity. The overall level of CAT was greatly decreased in all cell lines. However, the CMV promoter still expressed higher levels of CAT that the SV40 and mMT-1 promoters by 4.1- and 5.3-fold, respectively. Virus was rescued from the selected cell lines by transfection of the cells with helper plasmids. The transiently produced virus was used to infect fresh QT6 cells under conditions that would yield a single proviral insertion per cell. Infected cells will be assayed for NPT and CAT activity.

H 122 RETROVIRALLY MEDIATED GENE TRANSFER TO HUMAN HEMATOPOIETIC PROGENITORS, Phillip F.D. Hughes, Donna E. Hogge, Connie J. Eaves, and R. Keith Humphries, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3 Canada.
Although retroviruses can efficiently transfer foreign genes to hematopoietic progenitor cells, expression of the transferred gene has generally been poor, especially in the human system. We have investigated a number of ways of improving expression efficiencies, in particular, by using vectors containing internal promoters, and by adding growth factor supplements during the infection/selection period. Initial experiments have shown that the best results are obtained with high virus titres and co-cultivation techniques. Use of vectors that contain the neomycin resistance gene under the control of the thymidine kinase promoter have not shown the dramatic increase in efficiency expected on the basis of murine studies, however, inclusion of supplemental growth factors (GM-CSF and IL-1) during both the infection and selection period produces a marked difference. A 6-9 fold increase in efficiency has been observed in CFU-GM using the Tk-neo vector and growth factor supplementations. Using normal bone marrow progenitors, we routinely observe ≈10% CFU-GM resistant to high concentrations of G418 (1 mg/ml active drug). Selection with optimized G418 concentrations (but which still completely inhibit control colony formation) reveals gene transfer and expression of neo^R in at least 35% of CFU-GM. These improved efficiencies will now make it easier to introduce a variety of genes into human progenitors in order to provide further insights into hematopoietic stem cell differentiation and dynamics.

H 123 FACTORS IMPORTANT IN PRODUCING BETTER HELPER LINES FOR AMPHOTROPIC VIRAL VECTORS Marc Canteloube[*], Kimberly Carr[*], Jiing-Kuan Yee[+] and Douglas J. Jolly[*], [*]INSERM UNIT 33, Bicetre, France, and [+]Department of Pediatrics, University of California, San Diego.
Retroviral vectors have a number of advantages with respect to other methods of introducing genes into cells in culture or in animals, including their efficiency in placing one unrearranged copy of the incoming DNA in the host genome. However, there are significant problems which make many experiments difficult. We describe here our attempts to investigate two of these problems: a)the tendency of the helper lines to repair the defective helper genome and put out competent virus; b)The problem of obtaining high titres of amphotropic (able to infect cells from a range of species including mice) vector. We have therefore constructed two plasmids without LTR's or packaging signals, but which express the two open reading frames of the viral proteins (gag-pol and env). When these plasmids, pSVGP and pRSVENV were transfected into cells, these transfectants were able to behave as helper lines, but in a large number of experiments never gave us helper virus.

To try to increase titers, we have tried several different cell types with different helper configurations without much success. However, when we amplified the helper genomes cotransfected with selectable gene DHFR, by increasing methotrexate concentrations in culture, we observed an increase in the trancripts from the helper genomes. A comparison amongst such lines and others showed a good correlation between the levels of RNA and the efficiency of vector production. The level of helper genome RNA is therefore a limiting factor in the production of vectors.

H 124 RETROVIRAL-MEDIATED GENE TRANSFER LEADS TO REGULATED PRODUCTION OF HUMAN BETA GLOBIN CHAINS IN MICE, Stefan Karlsson[1], David Bodine[2], Leland Perry[1], Thalia Papayannopoulou[3], Arthur W. Nienhuis[2], [1]NINCDS and [2]NHLBI, National Institutes of Health, Bethesda, MD. [3]University of Washington, Seattle, WA.
We have previously shown that retroviral vectors can be used to transfer human globin genes into mouse erythroleukemia (MEL) cells resulting in high level production of human beta globin chains (PNAS, 84,2411-2415). A similar helper-free retroviral vector of higher titer (10^6 c.f.u./ml) has now been made using ψ2 helper cells. Mouse erythroleukemia cells that have been transduced with this vector express high levels of human beta globin RNA after induction with HMBA. Mouse bone marrow cells were infected and injected into lethally irradiated mice. Day 12 CFU-S derived foci were found to contain vector DNA in 80% of the foci when the infected donor marrow cells were preselected in G418 for 48 hours prior to transplantation. W/W^v mice with macrocytic anemia due to a deficiency of pluripotent hematopoietic stem cells have been transplanted with vector infected healthy murine bone marrow cells. An antibody against the human beta globin chain demonstrates production of human beta globin in blood erythroid cells of recipient W/W^v mice 4-5 weeks after transplantation using immunofluorescent staining.

H 125 RETROVIRAL MEDIATED TRANSFER OF GROWTH FACTOR GENES INTO MURINE AND HUMAN HEMOPOIETIC CELLS: A MODEL FOR LEUKEMOGENESIS, Karl Klingler[1,3], Christine Laker[1], Carol Stocking[1], Ursula Just[1], Norbert Kluge[1], Wolfram Ostertag[1], Elaine Spooncer[2], Makoto Katsumo[2], Michael Dexter[2], [1]Heinrich-Pette-Institut an der Universität Hamburg, 2000 Hamburg 20, F.R.G., [2]Paterson Institute for Cancer Research, Christie Hospital, Withington, Manchester M20 9BX, U.K., [3]present address: Walter and Eliza Hall Institute of Medical Research, Victoria 3050, Australia.

Retroviral mediated gene transfer, using amphotropic helper functions, into hemopoietic stem cell lines occurs with a very low frequency (10^{-4}) compared to fibroblasts. We constructed several retroviral vectors based on the myeloproliferative sarcoma virus (MPSV). With these vectors we obtained expression of M-CSF, GM-CSF, IL-3 and IL-4 genes in hemopoietic stem cell lines of the mouse and in human and mouse hemopoietic precursor cells. Some of those recipient cells normally require growth factors for their survival and proliferation. Introduction of certain growth factor genes leads to growth factor independence via secretory stimulation and subsequently to multi-step leukemogenesis. Introduction of some vectors induces specific differentiation.

H 126 TRANSFER AND EXPRESSION OF THE HUMAN ADENOSINE DEAMINASE (ADA) GENE IN ADA-DEFICIENT HUMAN T LYMPHOCYTES WITH RETROVIRAL VECTORS, Donald B. Kohn, Philip Kantoff, James Zweibel, Eli Gilboa, W. French Anderson and R. Michael Blaese, NCI and NHLBI, NIH, Bethesda, Md 20892.
Transfer of the human ADA gene into cells of an HTLV-1 transformed ADA-deficient human T line (TJF-2) by a retroviral vector (SAX) has been shown to result in the production of normal levels of ADA activity. We now report further characterization of the efficiency of ADA gene transfer and expression by SAX in ADA-deficient human T cells. Long-term cultures of non-transformed human T cells were established from patients with ADA-deficient SCID. These cells could be infected with the SAX vector by co-cultivation with vector-producing fibroblasts, leading to increased levels of ADA activity, similar to those produced in the TJF-2 cells. ADA gene transfer and expression in these cells by SAX was only modestly dependent on cell proliferation at the time of infection. To further examine the efficiency of ADA gene transfer and expression by SAX, TJF-2 cells which were infected by SAX, but not selected in G418, were cloned by limiting dilution. 6/27 (22%) of the clones of the T cells had acquired and were expressing the genes of the SAX vector. 1-3 copies of SAX/cell produced ADA in the range found in normal thymocytes and T cells. These results show that the SAX vector is capable of efficient transfer and expression of the human ADA gene in ADA-deficient human T cells.

H 127 HIGH EFFICIENCY GENE TRANSFER AND EXPRESSION IN NORMALHUMAN HEMATOPOIETIC CELLS WITH RETROVIRUS VECTORS, Pierre Laneuville, Axel A. Fauser and John E. Dick. Division of Hematology, Royal Victoria Hospital, McGill University, Montreal, Quebec and Division of Medical Genetics, Sick Children's Hospital, University of Toronto, Ontario, Canada.
Retroviral vectors containing the selectable bacterial gene for G418 resistance (neo) were used to demonstrate gene transfer into primary human bone marrow progenitor cells. In an attempt to obtain populations of cells where a high proportion of cells were expressing the neo gene we made several important modifications to earlier procedures. Normal donor bone marrow cells were infected with amphotropic replication defective neo virus by co-cultivation with PA317-N2 cells for 24 hours. Infection was performed with and without growth factors supplied by supernatants of the bladder cells carcinoma cell line 5637. Cells were subsequently exposed to high concentrations of G418 (2 mg/ml) for 48 hours in liquid culture to select for G418 resistant cells. Cells were plated in semi-solid methycellulose in the absence and presence of increasing doses of G418 to generate day 14 CFU-GM and BFU-E survival curves. The fraction of G418 resistant CFU-GM and BFU-E with G418 preselection increased 3-fold and approached 100%. Addition of 5637 conditioned medium had the effect of increasing the yield of G418R-CFU 2-3-fold. These results demonstrate that retrovirus vectors can be successfully used to transfer new genes at high efficiency into relatively undifferentiated progenitor cells in the human blood-forming system.

H 128 Retroviral vectors for the transfer of a mutated DHFR* cDNA into mouse bone marrow cells in vivo. Pierre M. Lehn, Paul D. Robbins and Richard C. Mulligan, Whitehead Institute, Cambridge MA 02142.
A cDNA encoding a mutated dihydrofolate-reductase (DHFR*) protein with a reduced affinity for methotrexate (MTX) can be used as a selectable marker in gene transfer experiments. Two different types of DHFR*-retroviral vectors were constructed. In the LTR-enhancerless vectors, the DHFR* gene is driven by an internal promoter (IP) (chicken beta actin or human H4 histone).Producer cell lines were generated by transfection of Psi-2 packaging cells and selection in 0.3 micromolar MTX. The highest viral titers (3T3 cfu/ml in presence of MTX) were obtained with the H4 construct, but they remained under 10*5. Several LTR-based vectors (with different enhancers in the LTRs) were also constructed. Viral titers as high as 5x10*6 could be obtained with a modified Zip-DHFR* vector containing the MPSV enhancer. Mouse bone marrow cells were infected with this MPSV vector and transplanted into lethally irradiated recipients. At 4 months post-transplant, the DHFR proviral structure could be detected by Southern in the spleen DNA of 6 out of 8 animals (copy number ranging from 0.1 to 0.5). However, no expression of the integrated provirus could be showed by Northern or SP6 analysis of total RNA from either spleen or bone marrow cells. In order to analyze if methylation was possibly involved in the proviral inactivation, a first investigation showed that the SmaI sites in the LTRs are not methylated. Extensions of this study are in progress, as well as the construction of more complex IP-based DHFR* vectors yielding higher viral titers.

H 129 **REGULATED EXPRESSION OF A HYBRID MOUSE/HUMAN β-GLOBIN GENE TRANSFERRED BY RETROVIRAL VECTOR,** **Chung-leung Li, Varavani J. Dwarki, Pamela Mellon and Inder M. Verma, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.**
A recombinant retrovirus, pN2MHβ8, containing a hybrid 5' mouse β major/ 3' human β-globin gene has been constructed in the N2 vector. It was anticipated that the use of 5' mouse DNA sequences will enable the regulatory sequences within this region to be recognized by species specific (if any) regulatory factors in mouse erythroid cells during erythropoiesis. We observed that the hybrid mouse/human β-globin gene, when introduced into murine erythroleukemia cells by infection with the recombinant retroviruses, showed a similar pattern of regulation. Furthermore, levels of expression was comparable to that of the the endogenous β major globin gene during DMSO-induced differentiation. The hybrid mouse/human β-globin containing retroviruses were also used to infect mouse normal bone marrow cells. Characterization of integration and expression in reconstituted mice would be presented.

H 130 GENE TRANSFER IN CANINE AND FELINE HEMATOPOIETIC PROGENITOR CELLS WITH RETROVIRAL VECTORS, C.D. Lothrop Jr, Z. Al-Lebban, M.A. Eglitis, W.F. Anderson and J.B. Jones. University of Tennessee, Knoxville, TN 37901 and Laboratory of Molecular Hematology, NIH, Bethesda, Maryland 10892.

The dog and cat are potentially excellent animal models to characterize retroviral vector mediated gene transfer for treatment of genetic diseases. Spontaneous genetic diseases, analogous to the human disorders affecting most organ systems have been described for the dog and cat. To characterize gene transfer in these animal models, we have used the retroviral vector N2 to transfer the bacterial Neo^R gene into hematopoietic progenitor cells from dogs and cats. Approximately 2-10% of fetal liver, fetal bone marrow and adult bone marrow day 7 CFU-GM were resistant to 2 mg/ml G418 after infection with the N2 retrovirus. At 2 mg/ml G418, there were no CFU-GM present in uninfected control cultures. Surprisingly, up to 50% of CFU-GM were resistant to 2 mg/ml G418 after N2 infection of neonatal hematopoietic progenitor cells. The neomycin phosphotransferase enzyme was detected in the G418-resistant CFU-GM, confirming G418-resistant CFU-GM expressed the Neo^R gene. Bone marrow from four adult cats was infected with the N2 vector and transplanted autologously. All 4 cats had a small but significant number (3-7%) of G418-resistant (2 mg/ml) CFU-GM 30 days after transplantation. One cat continues to have G418-resistant (2 mg/ml) CFU-GM after more than 220 days. These results demonstrate that the efficiency of retroviral infection and Neo expression is influenced by the developmental stage of the target hematopoietic cell and that retroviral vectors can be used for stable transfer of exogenous genes to feline hematopoietic cells in vivo.

H 131 USE OF RETROVIRAL VECTOR-MEDIATED GENE TRANSFER TO OBTAIN EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN MURINE HEMATOPOIETIC CELLS. Grant R. MacGregor, Frederick A. Fletcher, Kateri A. Moore, C. Thomas Caskey, John W. Belmont. Howard Hughes Medical Institute, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX.

Sixteen replication-defective retrovirus vectors which carry human adenosine deaminase (ADA) coding sequences were constructed to evaluate several functional parameters: (a) extended viral gag gene sequences in packaging of defective particles; (b) internal promoters in independent transcriptional units; and (c) altered 3' LTR's in packaging and expression. Each vector was transfected into the amphotropic packaging cell line PA317 or the ecotropic packaging cell line psi2 and stable transformant populations were tested for their ability to transduce ADA to Rat 208F target cells. Virus containing supernatants from populations which were positive in the initial screen were used to infect the ecotropic packaging cell line psi2. Individual psi2 clones were the tested for ADA transduction. These studies indicated that optimal production of virus was dependent on the region of gag 3' to the classical psi packaging sequence. Vectors with altered 3' LTR's function poorly in the production of virus. Retrovirus vectors with LTR, cFos, and HSVTK promoters were then used to infect mouse primary bone marrow cells. Virus transduction of the human ADA was assesed in in vitro colony forming cells (CFU-C) and spleen colonies (CFU-S) using enzyme assay and Southern analysis. Human ADA was expressed in progenitors infected with the viruses having LTR and HSVTK promoters but not in those with the cFos promoter. Seventeen of nineteen mice injected with a fully reconstituting dose of infected marrow showed expression of human ADA in their peripheral blood after 8 weeks. Three of nine mice continue to have human ADA in their RBC without reduction 102 days post transplant. These studies provide preliminary basic data on the feasibility of gene transfer as a therapy for ADA deficiency.

H 132 PSEUDOVIRION–MEDIATED GENE TRANSFER. Susan E. Gould–Fogerite, James J. Reston, Raphael J. Mannino. Albany Medical College, Albany NY 12208.

Pseudovirions, proteoliposomes containing the envelope glycoproteins of Sendai or influenza virus integrated in the lipid bilayer and DNA encapsulated within the aqueous interior, can mediate gene transfer to animals and to cells in culture. Sendai pseudovirions prepared by the protein cochleate method (Anal. Biochem. 145:15–28 [1985]) have been used to stably transform mouse C127 cells with a bovine papilloma virus–based plasmid at 100,000 times greater efficiency than calcium phosphate precipitation, with respect to the quantity of DNA needed to achieve maximal transfection. Preliminary data indicates stable transformation of primary hepatocytes at efficiencies similar to retro–viral mediated gene transfer.

Influenza pseudovirion mediated gene transfer to cells in culture achieved transient expression of DNA in 100% of cells with maximal expression occurring 6–12 hours after low pH activated fusion of cell–bound vesicles.

Pseudovirions containing a plasmid encoding polyoma virus early proteins were injected subcutaneously in the suprascapular region of newborn mice. Tumors which arose 6 to 7 months later were shown to contain the transferred DNA and express the polyoma pro–teins if encoded. Gene transfer to 50% of the mice in one experimental group was achieved.

H 133 CONSTRUCTION OF A SAFE PACKAGING LINE FOR USE IN GENE TRANSFER WITH RETROVIRAL VECTORS, D. Markowitz, S. Goff, and A. Bank, Columbia Univ., Depts. of Genetics & Development, Biochemistry & Molecular Biophysics, and Medicine, New York, New York 10032.
A major limitation to the use of retroviral vectors for gene transfer is the potential generation of replication-competent virus by a recombinational event between the helper virus and the replication-defective retroviral vector. To prevent recombination, a packaging cell line has been constructed by using portions of the Moloney murine leukemia virus in which the gag, pol, and env genes of the helper virus have been separated onto two different plasmids and the ψ packaging signal and 3' long terminal repeat have been removed. The plasmid containing the gag and pol genes and the plasmid containing the env gene were cotransfected into NIH-3T3 cells. One of the gag-pol and env clones that produced high levels of reverse transcriptase and env protein(GP+E-86) was able to package the retroviral vectors Δneo(at a titer of $1.7X10^5$)and N2(at a titer of $4X10^6$). A packaging line containing the intact gag-pol-env plasmid 3PO, had comparable titers($6.5X10^4$ with Δneo; $1.7X10^5$ with N2). Tests for recombinational events that might result in intact retrovirus showed no evidence for the generation of replication-competent virus. These results suggest that gag, pol, and env on different plasmids may provide an efficient and safe packaging line for use in retroviral gene transfer.

H 134 RETROVIRAL GENE TRANSFER AND EXPRESSION OF THE MULTIDRUG RESISTANCE GENE IN MURINE HEMATOPOIETIC CELLS. Jeanne R. McLachlin[1], Martin A. Eglitis[1] Kazumitsu Ueda[2], Elizabeth Lovelace[2], Michael M. Gottesman[2], W. French Anderson[1] and Ira Pastan[2], [1]Laboratory of Molecular Hematology, NHLBI,NIH; [2]Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892
We have used a retroviral vector to transfer a cDNA for the human multidrug resistance gene (MDR1) into mouse bone marrow cells. The MDR1 gene encodes a 170-kDa P-glycoprotein which serves as a drug efflux pump conferring resistance to a variety of drugs including colchicine, vinblastine or adriamycin. In a previous study, the same cDNA for the human MDR1 gene has been shown to express in both NIH 3T3 and human KB cells following transfer using retroviral vectors containing Moloney murine leukemia virus (M-MuLV) or Harvey murine sarcoma virus (Ha-MSV) LTRS (P.N.A.S. 84,3004,1987). In the present work we used the Ha-MDR1/A retroviral vector derived from Ha-MSV to introduce the full-length human cDNA for MDR1 into murine hematopoietic progenitor cells. Bone marrow cells were collected from DBA/2J mice and infected by cocultivation with either an ecotropic or amphotropic packaging cell line producing the HaMDR1/A vector. Infected cells were cultured for an additional 24 hours or plated directly into semi-solid culture for hematopoietic colony formation in the presence or absence of a selective drug. A high proportion of CFU-GM were resistant to colchicine at concentrations toxic to uninfected bone marrow cells from the same population. Further work will be done to evaluate the level of resistance in CFU-GM in vitro to other selective drugs.

H 135 **SMALL SEQUENCE CHANGES IN RETROVIRAL VECTORS CAN ADVERSELY AFFECT TITERS OBTAINED FROM PACKAGING LINES.** D. Muenchau, S. Freeman, J. A. Zwiebel, M. A. Eglitis, P. Kantoff, and W. F. Anderson. National Institutes of Health, Bethesda, MD 20892
A series of retrovirus-based vectors have been constructed that carry an SV40-promoted npt gene. These constructs were made using B2, a vector that has sequences identical to the N2 vector but is constructed in the pGEM4 plasmid. The LTR fragments from N2 were inserted into the EcoRI and HindIII sites of the pGEM4 polylinker. It thus differs from N2 in the region between the LTRs by containing the pGEM4 polylinker sequence. The B2 homolog of N2 (B2N) with npt driven off the LTR has the same in vitro titer but different in vivo expression characteristics as N2. Both vectors give maximum $G418^r$ particle titers near $5X10^6$ and express the npt protein in the mouse spleen focus assay. However, initial characterization of B2 derivatives carrying an SV40-npt fragment shows maximum $G418^r$ particle titers of $2X10^5$ in psi2 while either low titers (near $3X10^4$) or no titers are observed on the PA317 packaging line. These results have been obtained using pB2SVN (npt driven by SV40 promoter) and G2N (rat GH driven off the LTR and SV40 driven npt). These observations suggest that the SV40-npt fragment interferes with virus transcription or expression of integrated npt vector sequences. Earlier studies with N2 showed that npt is expressed from a transcript generated by a cryptic splice acceptor near the npt gene (J. Virol. 61:1647-1650 1987). We report the results of analyses of RNAs obtained from the psi2 and PA317 cell lines containing integrated vector sequences, including measures of the relative abundance of different transcripts.

H 136 RECOMBINANT RETROVIRUS AS A TOOL TO INTRODUCE AND EXPRESS FOREIGN GENES IN SOMATIC AND GERM CELLS.
J.F. Nicolas, C. Bonnerot, D. Rocancourt, Génétique cellulaire, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15 ; P. Briand, G. Grimber, Biochimie génétique, Hôpital Necker, 149 rue de Sèvres, 75015 Paris, France.
A basic problem in development is the relationship between the lineage of a cell and its specification. In mammals, this aspect is difficult to study because of the lack of knowledge of lineages. Methods have been developed which combine the advantages of a reporter gene to detect expression in single cells and of recombinant retrovirus to introduce it into cells. Two different LacZ gene have been used. The first one express a β-galactosidase localized in the cytoplasm of transformed cells. The second one express a β-galactosidase which is targeted to the nucleus by fusing it to a location signal. Both markers can be histochemically detected in single cells, even on histological sections. Nls β-galactosidase is localized at the nuclear periphery of all cell types studied including 2-cell embryos. These reporter genes have also been used to demonstrate that, in the zygote, the time at which expression of introduced DNA starts, corresponds to the stage of activation of the embryonic genome. At that time, a specificity in the utilization of promoters already exists : the LTR of MoMLV and the promoter of IL-2 are silent, but ubiquitous promoters (β-actin, HPRT, HMG) are active. Unexpectedly, the H-2K^b promoter is active in 2-cell embryos, although in EC cells, the same construct is inactive. Stocks of 10^4 to 10^6 LacZ recombinant retroviruses have been produced and used to examine the lineage of tissues by infecting embryos *in vitro* and *in utero*.

H 137 DEVELOPMENT OF AN SV40-PSEUDOVIRAL VECTOR FOR SOMATIC GENE THERAPY,
Ariella Oppenheim, Nava Dalyot, Aviva Peleg and Eliezer A. Rachmilewitz, Department of Hematology, Hadassah University Hospital, Jerusalem, Israel, 91120.
As the first step in the development of a procedure for gene therapy, we have developed a novel vector for efficient introduction of foreign DNA into human hemopoietic cells. The vector carries the SV40 origin of replication and prokaryotic plasmid sequences for propagation in bacteria. The plasmid is encapsidated in COS cells as an SV40 pseudovirus with a helper that provides the SV40 late proteins. High titer stocks can be readily prepared. The plasmid is then transmitted by viral infection into the target human hemopoietic cells, including erythroid cells, at a very high efficiency. The vector is particularly suitable for studying expression of cloned genes, for the following reasons: a. Minimal vector sequences are required for the preparation of pseudovirions (200bp), therefore potent regulatory signals that may interfere in studies on the control of expression of the cloned gene can be excluded. b. Due to its high efficiency, the vector can be used for transient expression experiments in the human hemopoietic cells. c. The same gene construct can be investigated with and without gene amplification, depending whether it is encapsidated with a wild-type SV40 as a helper or with a T-antigen negative helper. We plan to use it for rapid screening of new, manipulated beta-globin constructs, in order to locate and investigate potential regulatory sequences. We are currently starting experiments utilizing this vector for studies on expression of cloned human globin genes and in gene targeting experiments.

H 138 POTENTIAL CORRECTION OF GENETIC DEFICIENCIES WITH GENETICALLY MODIFIED NORMAL DIPLOID FIBROBLASTS.
T. D. Palmer, R. A. Hock, W. R. A. Osborne, A. R. Thompson and A. D. Miller. Fred Hutchinson Cancer Research Center, 1124 Columbia Street, and University of Washington, Seattle, Washington 98104.
Retroviruses carrying therapeutic genes can be introduced into normal human fibroblasts with high efficiency. In vitro, these fibroblasts produce significant amounts of active therapeutic product. To explore the potential of fibroblast mediated therapy, retroviruses were constructed that contained a human adenosine deaminase (ADA) cDNA or a human blood clotting factor IX cDNA. We have demonstrated that in vitro, fibroblasts isolated from an ADA-deficient patient and infected with the ADA vector produced 12-fold more ADA enzyme than fibroblasts from normal individuals and were able to rapidly metabolize exogenous metabolites which are responsible for the severe combined immunodeficiency syndrome of ADA deficiency. In fibroblasts infected with the factor IX vector, factor IX was produced at >250ng/10^6 cells/day. Greater than 95% of this factor IX was active by its ability to complement factor IX deficient plasma in clotting assays. In on going experiments to determine the most effective route of reintroduction, rat fibroblasts were able to act as a skin substitute when introduced in a collagen matrix to open skin wounds, and dextran beads coated with fibroblasts form vascularized, organ-like masses when introduced into the peritoneal space. The distribution of therapeutic product in these two models is currently being explored.

H 139 ANALYSIS OF ACTIN GENE PROMOTER FUNCTION IN RETROVIRAL VECTORS. Christos J. Petropoulos, Michael Rosenberg, and Stephen H. Hughes. BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701.
We are interested in identifying regions of the chicken skeletal muscle α-actin and non-muscle β-actin genes that are responsible for controlling tissue-specific actin gene transcription. To do so, portions of actin gene 5' flanking regions have been linked to the bacterial chloramphenicol acetyl transferase gene (CAT). We have employed pronuclear microinjection techniques to produce mouse lines carrying actin/CAT transgenes and we are characterizing the level of CAT activity produced in various tissues of transgenic mice. Our preliminary findings with transgenic mice suggest that 200 bp of 5' flanking sequences are sufficient for appropriate tissue-specific expression of the chicken skeletal muscle α-actin gene. We would also like to determine whether cellular promoters (such as the actin promoters) remain functional and retain their tissue specificity when they have been embedded in retroviral vectors and reintroduced into cells. For these studies we have introduced various actin/CAT constructs into primary chicken myoblast and fibroblast cultures, using the replication competent avian retroviral vector system developed in our laboratory (Hughes et al., J. Virol. 61:3004, 1987). Our data indicate that both the muscle α-actin and non-muscle β-actin gene promoters remain functional when reintroduced into primary chicken cell cultures using retroviral vectors. Ongoing *in vitro* studies are directed at determining whether actin promoters within integrated proviruses exhibit appropriate tissue specificity. Research sponsored by the National Cancer Institute, DHHS, under contract No. N01-CO-74101 with Bionetics Research, Inc.

H 140 TARGETTING AND TISSUE-SPECIFIC EXPRESSION OF Mo-MuLV DERIVED RETROVIRUSES, Pierre Roux, Philippe Jeanteur and Marc Piechaczyx, Laboratoire de Biologie Moleculaire-USTL - Place E. Bataillon - 34060 Montepellier cedex - France. The targetting of Mo-MuLV recombinant retroviruses as well as the tissue-specific expression of inserted genes were addressed. Bi-specific antibody complexes directed against the *env* protein and cell membrance proteins were used to modify the tropism of ectropic Mo-MuLV derived retroviruses. Substitution of LTR promoter and/or enhancer by tissue-specific promoters and/or enchancers as well as the insertion of characterized promotomers in suicide retroviral vectors were performed to control the expression of genes cloned in Mo-MuLV derived retroviruses. Our results will be presented and discussed at the congress.

H 141 EFFICIENT EXPRESSION IN EMBRYONAL CARCINOMA CELLS OF A RETROVIRUS VECTOR CARRYING THE HUMAN β-ACTIN PROMOTER, Ian B. Robson and Alan Bernstein, Mt. Sinai Hospital Research Institute, Toronto, Canada, M5G 1X5.
We set out to build a retrovirus vector from which transduced genes would be efficiently expressed in embryonal carcinoma cells, and in transgenic mice generated by embryo infection. Vectors of two designs were constructed, i) the human β-actin promoter driving the marker gene *neo* was inserted in a Moloney-MuLV based vector backbone, ii) the HSV-*tk* promoter driving the *neo* gene was inserted in a "wings-clipped" virus backbone, which generates a provirus lacking retrovirus LTR enhancer sequences. The β-actin:neo virus formed G418^r colonies with equal efficiency in F9 and NIH3T3 cells. The wings-clipped HSV-tk:neo virus formed G418^r F9 colonies approximately 100 fold less efficiently than G418^r 3T3 colonies. An HSV-tk:neo virus with intact LTRs behaved identically to the wings-clipped derivative. F9 and 3T3 cells were infected at high multiplicity with the β-actin:neo virus and grown non-selectively for several days. The F9 and 3T3 cell populations were shown by Southern blot to have equal numbers of proviruses. Preliminary evidence from Northern blots and RNA protection experiments shows that the F9 and 3T3 cell populations contain equal amounts of neo RNA, and that this neo message is expressed from the β-actin promoter. Transgenic mouse lines have been generated by infection of 2- or 8-cell stage CD1 mouse embryos with the β-actin:neo virus. These are currently being analyzed for expression of the transduced *neo* gene.

H 142 TRANSFER AND EXPRESSION OF THE HUMAN IL-2 RECEPTOR GENE IN MURINE FIBROBLASTS AND T-LYMPHOID CELLS USING RETROVIRAL VECTORS, Barbara Seliger, Gracia Kruppa, Klaus Pfizenmaier, Clinical Research Group, Max-Planck-Society, Goßlerstr.10d, 3400 Göttingen, FRG
In order to compare the suitability of different retroviral vectors for gene transfer into murine target cells of distinct tissue origin, we used the human IL-2 receptor gene as a model. We have employed both double expression (DE) vectors and vectors with internal promoters (VIP), carrying the neomycin resistance gene, to introduce and express the human IL-2R gene in murine fibroblasts and T-cell lines. First, the IL-2R cDNA was inserted into the DE vector N2. In this vector the expression of the IL-2R gene is controlled by LTR and is thus limited to compatible target cells. Second, to circumvent the problem of restricted tissue specificity we used retroviral vectors containing internal promoters. In these vectors the neo^{R}gene is expressed from the LTR, whereas expression of the human IL-2R cDNA is controlled by either the Tk or SV40 promoter. After transfection of the different constructs into NIH3T3 fibroblasts neo^R clones were isolated, containing intact copies of neo IL-2R DNA sequences. Northern blot analysis showed that the neo IL-2R clones express high levels of steady state IL-2R mRNA. Furthermore, immunofluorescence analyses demonstrate expression of Tac antigen on the cell surface of several neo^R transformants. Our data show that VIP vectors are superior to DE vectors with respect to both gene transfer efficiency and high expression of the gene product in question. Using BW5147 as a model to study the efficiency of gene transfer in cells of hematopoietic origin, we found that cocultivation of BW5147 cells with virus-producing neo IL-2R transformants resulted in a high infection rate of BW5147, indicating the suitability of these vectors for transfer and stable expression of eukaryotic genes into lymphoid cells.

H 143 PURIFICATION OF MURINE HEMOPOIETIC REPOPULATING STEM CELLS USING A NOVEL TWO-COLOR STAINING PROCEDURE AND FLUORESCENCE ACTIVATED CELL SORTING. Stephen J. Szilvassy, Peter M. Lansdorp, R. Keith Humphries, Allen C. Eaves and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., Canada.
Primitive hemopoietic colony-forming cells display low levels of Thy-1 antigen and high levels of class I MHC antigens on their cell surface. To study the characteristics of marrow stem cells with long-term (3-6 months) myeloid and lymphoid repopulating ability, we have developed a novel, one step purification procedure based on differential Thy-1 and class I MHC antigen expression and forward and perpendicular light scatter properties. Marrow cells are stained indirectly with avidin-FITC and R-phycoerythrin following primary incubation with biotinylated anti-Thy-1 monoclonal antibody and a tetrameric complex of an anti-H-2K^b monoclonal (IgG_1) antibody coupled to an anti-R-phycoerythrin monoclonal (IgG_1) antibody by 2 monoclonal rat anti-mouse-IgG_1 antibodies. By appropriate gating, a suspension containing >1% CFU-GEMM (macro) and >10% day 9 CFU-S can be obtained in this way from normal marrow. Day 4 5-FU marrow yields a suspension containing 1% CFU-GEMM (macro) and 20% day 12 CFU-S. These sorted 5-FU male marrow cells were also transplanted into syngeneic, lethally irradiated female recipients together with a 100-fold excess of female cells previously compromised in their repopulating ability by serial marrow transplantation. Southern analysis with a Y-specific probe showed dominance of male DNA in marrow, spleen and thymus 3-6 months later in recipients given as few as 10^3 sorted male cells. This sorting procedure should be useful for future experiments where introduction of genes into defined purified stem cell populations is desirable.

H 144 RETROVIRUS TAGGED MURINE CELL LIBRARIES USEFUL FOR GENE TRANSFER TO HUMAN CELLS, Terry L. Timme, Carla M. Wood and Robb E. Moses, Baylor College of Medicine, Houston, TX 77030.
We have infected murine cells with replication-defective retroviruses containing dominant selectable markers to generate libraries useful for genetic complementation of human cells defective in DNA damage-processing. Several different viral constructs have been packaged in either amphotropic or ecotropic retrovirus packaging cell lines to produce virus with titers of 10^4 to over 10^6. Retrovirus packaged in either PA12 or PA317 cells as amphotropic virus was less suitable than that packaged in $\psi 2$ cells as ecotropic virus due to production of a variable but significant level of replication-competent virus. We have infected NIH3T3 cells with ZIPNEO retrovirus and pooled about 5 x 10^4 independent G418^R colonies as a library. Similarly, over 7 x 10^4 G418^R colonies of HGPRT$^-$ murine CAK cells have been pooled following retroviral infection. We are thus able to monitor murine DNA transfer by selecting in G418 after transfection or electroporation with either purified DNA or isolated chromosomes, or by murine micronuclear fusion to human cells. Current efforts are directed to evaluating G418^R human cells cytogenetically and by Southern blot analysis for mouse DNA and for complementation of the DNA damage processing defect in xeroderma pigmentosum and Fanconi anemia cells. Supported by USPHS CA37860.

H 145 EXPRESSION OF GENES TRANSFERRED INTO HEMATOPOIETIC CELLS BY ELECTRO-PORATION. Frances Toneguzzo and Armand Keating, EG&G Biomolecular Watertown, MA 02172, and Toronto General Hospital, University of Toronto, Toronto, Canada M5G 2C4.
Characterization of DNA transfer showed that the levels of transient and stable gene expression in hematopoietic cells increased when increasing DNA concentrations were used during electroporation. Southern analyses, however, showed that even at high concentrations, DNA transferred by electroporation was preferentially integrated in low copy number. Although the site of insertion of foreign DNA into the host cell chromosomes was random, integration occured exclusively at the ends of the linear molecules used for transfection. Restriction endonuclease digestion indicated that the processes of transfer and integration proceed in the absence of DNA rearrangements. Following electroporation of human bone marrow, genes regulated by SV40 early region sequences were expressed both in granulocytic and erythroid progenitor cells as well as in their differentiated progeny. To examine the effect of different regulatory sequences on the expression of genes in hematopoietic cells, vectors containing the CAT gene under the control of different viral and cellular enhancers were transfected into human marrow and the levels of CAT expression quantified. Our results indicate a differential effect of regulatory sequences on gene expression in subpopulations of cells isolated from human bone marrow.

H 146 SOMATIC CELL GENE THERAPY: USE OF RECOMBINANT RETROVIRUS INFECTED SKIN FIBROBLASTS, Daniel St. Louis and Inder M. Verma, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.
The concept of human gene therapy involves the introduction of a functionally active gene into the somatic cells of an affected individual to correct the defect. We have explored the possibilities of using skin fibroblasts to efficiently deliver genes into animals. Mouse primary skin fibroblasts were infected with a recombinant retrovirus containing human factor IX cDNA. Bulk infected cells capable of synthesizing and secreting biologically active human factor IX protein were embedded in collagen and the implant grafted under the epidermis. Sera from the transplanted mice contains human factor IX protein for at least 10-12 days. The loss of immunoreactive human factor IX protein in the mouse sera is not due to the rejection of the graft. Instead the mouse serum contains mouse anti-human factor IX antibodies. We suggest that retroviral-infected primary skin fibroblasts offer a viable approach to somatic cell gene therapy.
Supported by NCI.

H 147 THE NEW EXPRESSION SYSTEM BASED ON AUTONOMOUSLY REPLICATING SEQUENCES OF THE MOUSE. Ulrich H. Weidle*, P. Buckel* and F. Grummt. *Boehringer Mannheim GmbH, 8122 Penzberg and Institute of Biochemistry, University of Wurzburg, 8700 Wurzburg, FRG.
The new expression system is based on a truncated thymidin kinase gene (one Spl binding site of the promotor is removed) and an autonomously replicating sequence (ARS) of the mouse. As a model system we discuss the expression of human-tissue type plasminogen activator cDNA driven by the SV40 early promotor after DNA mediated gene transfer into thymidin kinase deficient mouse fibroblasts. After selection of stable mouse transformants several morphologically different cell lines could be distinguished. Expression levels up to 8 µg/plasminogen activator/ml/10^6 cells/24 h were noted. We have determined the copy of number of the episomal plasmids by Southern blotting of Hirt supernatants. A tenfold decrease in the number of colonies per equivalent amount of DNA and cell number was noted when the expression cassette for plasminogen activator was placed onto the vector. This finding is correlated with the copy number per cell of the two constructs.

H 148 IN VIVO GROWTH HORMONE PRODUCTION BY CELLS CARRYING RECOMBINANT RAT GROWTH HORMONE GENES, James A. Zwiebel, Scott Freeman, Daryl D. Muenchau, Phillip W. Kantoff, John A. Thompson, and W. French Anderson, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892.

Two retroviral vectors, pLPGHL and pG2N, carrying recombinant rat growth hormone genes were used to transfect PA317 cells, a murine fibroblast line capable of producing infectious helper-free retroviral particles. pLPGHL and pG2N-containing clones, producing approximately 300 ng/10^6 cells/24 hrs and 50 ng/10^6 cells/24 hrs of rat growth hormone, respectively, were implanted by intraperitoneal or subcutaneous injection into hypophysectomized female Fischer rats. Before these cells were implanted, rat growth hormone could not be detected in the blood of the animals. Following the implants, rat growth hormone could be detected by radioimmunoassay in the blood within 4 hours; the level peaked within one day and declined to undetectable levels one week later. The administration of dexamethasone to the animals did not prevent the decline of in vivo growth hormone production, nor could the implanted cells be retrieved by explant culture of peritoneal tissue. To determine whether the decline is due to short in vivo survival of the implanted cells or to inactivation of the growth hormone gene, cells with the recombinant rat growth hormone gene were suspended in alginate beads and placed into the peritoneal cavities of hypophysectomized animals. Viable cells were easily retrieved 2 weeks after implantation and blood growth hormone levels persisted for one week before declining. This model system will allow the study of the expression of recombinant genes in transplanted cells.

Transgenic Animals

H 200 Male sterility in mice transgenic for a MUP promoter-HSVtk reporter gene.

Raya Al-Shawi, Joanne M. Meechan, Cheryl Jones, J. Paul Simons* & John O. Bishop.
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The mouse major urinary proteins (MUPs) are synthesized primarily in liver, and also in salivary, mammary and tear glands. In liver, male mice synthesize five times as much MUP as female mice; in females, male levels of MUP expression are induced on treatment with testosterone.

We have been studying the promoter region of one MUP gene (BS-6) by reintroducing it into mice. The promoter region of BS-6 was fused to the coding region of the HSV thymidine kinase gene, and the recombinant gene was microinjected into fertilized mouse eggs. In several lines of transgenic mice, HSVtk mRNA and HSVtk enzyme activity are detected in the liver, but not in the salivary glands, tear glands, brain, kidney or spleen. Expression of the transgene in the livers of female mice is elevated after treatment with testosterone. Unexpectedly, the transgene is expressed in the preputial glands and testes of male transgenic mice. The testes are the major site of expression in male transgenic mice, this expression results in sterility.

H 201 COOPERATIVE EFFECTS OF THE RAS AND THE MYC ONCOGENE ON DISTURBED DIFFERENTIATION BUT NOT ON TRANSFORMATION OF MAMMARY EPITHELIAL CELLS IN TRANSGENIC MICE. A. Andres, C. Schoenenberger, F. Flueckiger, M. Lemeur*, P. Gerlinger*, M. van der Valk+ and B. Groner. Ludwig Insitute for Cancer Research, Inselspital,3010 Berne, Switzerland, *CNRS, Strasbourg, France, + The Netherlands Cancer Institute, Amsterdam, The Netherlands.

We established transgenic mice bearing the murine c-myc oncogene under the control of the mammary gland specific and hormone dependent promoter of the murine Wap gene. Compared to previous studies on transgenic mice bearing the Wap-ras oncogene, we observe a different tumorigenic potential of the myc oncogene in the mammary epithelium. The ras oncogene was not efficient in transformation of the mammary epithelial cells, whereas 80% of the Wap-myc expressing females develop mammary tumors. These tumors show expression of the Wap-myc gene, the endogenous Wap and casein genes independent of lactation. In contrast to ras, myc seems to be able to overcome the hormonal control of the mammary epithelial cells. To study if this effect of myc is able to cooperate with the ras oncogene, we established transgenic mice bearing both, the Wap-ras and the Wap-myc oncogene. Both genes are coexpressed in the mammary glands during pregnancy and lactation. Compared to the Wap-myc expressing females we find neither an increase in tumor incidence nor a shorter tumor latency in the double transgenic animals. Coexpression is rather counteracting than cooperative in transformation of differentiating mammary epithelial cells. Each oncogene alone affects the differentiation of the mammary epthelium, predominantly reflected by a reduced expression of the Wap and β-casein genes. Cooperative effects of myc and ras on the differentiation are found in the double transgenic animals. Mammary glands of these animals show an abnormal morphology and almost no expression of milk specific protein genes.

H 202 IMMORTALIZATION OF HUMAN FIBROBLASTS, Ashutosh Banerjee, Eri S. Srivatsan, Helen Kim and William F. Benedict, Division of Ophthalmology, Childrens Hospital of Los Angeles, Los Angeles, California.

The chromosomal rearrangements seen in non-tumor cells of certain patients with retinoblastoma and Wilms' tumor (a deletion of 13q14 and 11p13 respectively are not themselves sufficient for carcinogenesis, but these non-tumor cells which carry the same chromosomal abnormalities as in tumor cells may provide excellent target cells to understand the mechanisms of human tumor formation. In our preliminary study, we obtained morphologically transformed aneuploid cells after irradiating fibroblasts derived from a patient with a non-deletion form of retinoblastoma although these cells were not tumorigenic in nude mice. This led us to believe that the cellular immortality may be a critical event in human neoplastic transformation.

We therefore focused our attention in immortalizing the fibroblasts derived from patients with deletion and non-deletion forms of retinoblastoma as an initial step for malignant transformation. We transfected these fibroblasts with a plasmid vector (pSV_2gpt) using the polybrene-DMSO technique. Transfecteed cell strains (LA-RB69F and LA-RB 110F) formed well defined foci, grew in soft agar (LA-RB 110F), and contained vector sequences integrated into the genome. Both strains have altered morphology and the strain LA-RB 110/gpt, after a short period of crisis, apparently has acquired immortality. Both transfected strains showed multiple chromosome rearrangements and heterogenous chromosome numbers. We envisage that these studies would develop into a potential in vitro model system in elucidating the chromosomal and molecular mechanisms of human transformation and hopefully changes responsible for tumorigenicity.

H 203 THE TRANSCRIPTION OF THE T CELL RECEPTOR β -CHAIN GENE IS CONTROLLED BY A DOWNSTREAM REGULATORY ELEMENT, Anton Berns, Michael Steinmetz* and Paul Krimpenfort, Division of Molecular Genetics of the Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; *Central Research Unit, Hoffmann-La Roche, Basel, Switzerland.

To characterize the cis-acting control elements involved in the expression of the T cell receptor α- and β-chain we generated several series of transgenic mice harboring functionally rearranged T cell receptor α- and β-chain genes, isolated from an $H\text{-}2D^b$ restricted cytotoxic T cell clone reactive to the HY antigen. Since in the initial experiments the TCRβ gene was not expressed in the founder mice we generated a series of transgenic TCRβ transgenic mice with different extensions of 5' and 3' sequences. Transcription analysis of transgenic mice carrying these clones showed that a 5 kbp fragment located downstream from the poly-adenylation signal of the C β2 region is indispensable for transgenic expression of TCRβ. Expression in transgenic mice was largely confined to T cells and reached levels comparable to normal functional T cell clones. A low level of expression was observed in B cells. Analysis of these mice showed that all T cells expressed the transgenic β-chain. Expression of the β-chain resulted in a suppression of rearrangement of the endogenous β-chain at an early stage: Partial D β 1-J β 1 rearrangements are found while VDJ rearrangements are not seen. The location of enhancer sequences in the fragment downstream from C β 2 was further defined by transient CAT-assays. A lymphoid-specific enhancer activity, in strength comparable with that of the Moloney MuLV LTR, was found to reside in a 550 bp fragment located 5 kbp downstream from C β-2.

H 204 STUDIES ON EXPRESSION FROM CARBONIC ANHYDRASE (CA) PRESUMPTIVE REGULATORY REGIONS IN TRANSGENIC MICE, AN APPROACH TO GENE THERAPY IN CA II DEFICIENT MICE. Arturo Bevilacqua, Robert Erickson, Patrick Venta and Richard Tashian, University of Michigan, Ann Arbor, Michigan 48109-0618.

We have developed 6 transgenic lines of mice with constructs containing presumptive 5' regulatory regions of CA II. One line combining 1.1 kb of mouse 5' region with bacterial chloramphenicol acetyl transferase (CAT) has weak expression of CAT in heart (normally low expressing) and no expression in blood, kidney and other tissues normally expressing large amounts of CA II. One line and one dead transgenic mouse (which didn't survive to found a line) with 1.4 kb of human 5' sequences also showed inappropriate expression of CAT and lack of expression in expected tissues. Four other transgenic lines are currently being tested. These constructs, whose CA insert ended at the start codon, contain only one-half of a GC rich island which flanks the first exon. A current construct contains the mouse 5' region through the first intron and joins to a human cDNA, thus including all of the GC rich island. Its expression will be studied at the mRNA level in mice containing an endogenous CA II gene. We have also injected CA II null zygotes which normally develop into mice runted with renal tubular acidosis, an excellent model of the human deficiency state. Studies of CA II expression at the protein level, and possible correction of the disease will be studied in these mice. Constructs including 3' flanking sequences and other exons will be made if appropriate expression does not occur with the complete GC rich island.

H 205 GENETIC ANALYSIS OF HOX 3.1, A MOUSE HOMEO-GENE, Philippe Brûlet, Hervé Le Mouellic, Yvan Lallemand and Patrice Blanchet, Unité de Génétique cellulaire, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

Our study of a mouse homeo-gene, Hox 3.1, has led to the characterization of the coding sequence and the distribution of transcripts in mouse embryos from 7.5 to 14.5 days p.c. by in situ hybridization experiments. Transcription is first detectable in the whole posterior part of the 8.5 day embryo, without tissue specificity. This distribution follows gradual location and tissue restrictions. Thus, by day 10.5, transcription is concentrated in the median part of the embryo, in the somitic mesoderm and the neural tube, where it is the most active in the ventral horns. By day 12.5 transcripts are only detectable in the neural tube, mostly at the level of the heart. The pattern of transcription of Hox 3.1 is suggesting a regionalizing role during embryogenesis.
Gene transfer into the mouse egg appeared convenient for the genetical study of Hox 3.1. To obtain the equivalent of a dominant mutation, we choosed to place the Hox 3.1 gene under the influence of a strong and ubiquitous promoter in transgenic mice. The DNA molecule was constructed with the RSV promoter upstream to the minimal cDNA sequence containing the ORF. It was micro-injected into fertilized eggs which were furtherly re-implanted in mice. Analysis is in progress.

H 206 INTEGRATION AND GERM-LINE TRANSMISSION OF FOREIGN GENES IN TROUT AFTER INJECTION INTO THE EGG CYTOPLASM, Daniel Chourrout, René Guyomard, Christine Leroux, Francine Pourrain and Louis-Marie Houdebine, laboratory of Fish Genetics, INRA, 78350, Jouy-en-Josas, France.

Fish external fertilization and high fecundity greatly facilitate gene transfer experiments. However, pronuclear injection, as well as the use of genetically engineered retroviruses are not yet possible in these animals. For that reason, we examined the potential efficiency of injecting foreign DNA into the cytoplasm of fertilized eggs, before the first cleavage. Each egg received 20 million copies of one of five plasmids, bearing hGH or rGH genes (or cDNAs) coupled with various promoters (mouse MT1 or H2K, SV40 early gene, LTR of MMTV). 60 % of the eggs gave rise to adult fish, and more than 50 % of them kept the foreign DNA. Variations of the average copy number were found between different individuals, and also between different tissues of most fish. Southern blots indicated frequent head-to-tail concatemers, some of them being episomal, and most of them being most likely integrated in the trout genome. Eight of fifteen males analyzed transmitted the foreign DNA to about 20 % of their offsprings (5 to 30 %), in which putative junction fragments were easily detected (with no episomal exogenous DNA). These data confirmed the integration of the plasmids in the parental genome in a mosaic distribution, this integration probably happening at early development stages. No growth hormone could be detected in all fish examined : this may be due to the poor activity of mammalian promoters in fish cells. The method used here thus permits the production of fourty transgenic adults per day of injection, with a very efficient transmission of the foreign DNA to the offsprings.

H 207 A FUSION CONSTRUCT OF THE MOUSE ALPHA AMYLASE-1A GENE PROMOTER AND SV40 TUMOR ANTIGEN GENE EXHIBITS A NOVEL TISSUE SPECIFICITY OF EXPRESSION AND TUMOR PRODUCTION IN TRANSGENIC MICE, Niles Fox, Rosanne Crooke, Lih Hwa Hwang, Barbara B. Knowles and Davor Solter, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Transcription of the mouse alpha amylase-1^a gene occurs from two different promoters. The stronger upstream promoter is active only in the parotid gland while the weaker downstream promoter is active in the liver, parotid and pancreas. As a means of studying the sequences responsible for the tissue specificities of these promoters, we have developed mice transgenic for fusion gene constructs containing either promoter region fused to the SV40 tumor antigen gene (constructs: p1.3T: strong promoter, p600T: weak promoter). Interestingly, while neither construct was expressed or induced the formation of tumors in the parotid, liver or pancreas, a high incidence of tumors arose in the brown adipose tissue (BAT) of mice transgenic for the p600T construct. By light and electron microscopy these tumors appeared as brown fat tumors or "hybernomas" and expressed abundant T-antigen. BAT cells as well as white adipose cells in the surrounding tissue as well as in other areas where adipose tissue is found, also showed varing degrees of nuclear and cellular atypia whereas all other tissues appeared normal. Preferential expression of p600T in the adipocyte cell lineage is presently under investigation.

H 208 EXPRESSION OF HUMAN TPA IN THE MILK OF TRANSGENIC ANIMALS, Katherine Gordon, Christoph W. Pittius#, James Vitale, Lothar Hennighausen#, Eric Lee*, Edward Nicols, and Heiner Westphal*, Integrated Genetics, 31 New York Avenue, Framingham, MA 01701, #Laboratory of Biochemistry and Metabolism, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD 20892, *Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
We have produced a series of transgenic mice which contain a mammary-specific expression cassette in order to target production of tissue plasminogen activator to the lactating mammary gland. The fusion construction contained the promoter from the murine whey acid protein gene and the secretion signal of the TPA gene. Human TPA was secreted into milk in all transgenic lineages which expressed product, though there was much variability among the different lineages. There was no correlation which could be drawn between DNA copy number and level of expression. In one lineage which was characterized in detail, accurate distribution of transcripts was seen, compared to the distribution of endogenous RNA coding for whey acid protein. The fusion expression vector was subject to accurate hormonal regulation since the expression level was vastly reduced in the mammary gland of virgin female sibling animals. The protein produced in the milk of transgenic mice appeared to comigrate with the two-chain form of authentic, recombinant human tissue plasminogen activator.

H 209 USE OF THE ADENOVIRAL MAJOR LATE PROMOTER TO DIRECT THE TRANSCRIPTION OF THE RAT ORNITHINE TRANSCARBAMYLASE (OTC) IN TRANSGENIC MOUSE, Michèle Minet Thurriaux, Catherine Cavard, Gisèle Grimber, Jean-François Chasse, Myriam Bennoun, Nathalie Dubois, Pascale Briand. LBG Necker hospital Paris FRANCE.
The rat OTC cDNA sequence under the control of the adenovirus major late promoter has been introduced into fertilized eggs. Integration and expression of the introduced sequence was detected in a transgenic animal which has developped from these eggs. The levels of the OTC mRNA and enzyme activities in various tissues of the transgenic animal have been determined and compared with those obtained in a transgenic animal in which the same cDNA sequence is under the control of the SV40 early promoter.

H 210 POSITION INDEPENDENT HIGH LEVEL EXPRESSION OF THE HUMAN BETA-GLOBIN GENE IN TRANSGENIC MICE, Frank Grosveld, Greet Blom van Assendelft, David R. Greaves and George Kollias, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.
We have constructed a "mini" locus that contains the 5' and 3' flanking regions of the human beta-globin locus and the beta-globin gene. These regions are characterized by erythroid specific DNaseI superhypersensitive sites and are normally localized approximately 50kb 5' and 20kb 3' from the beta-globin gene. This "mini" locus is expressed tissue-specifically in transgenic mice at a level directly related to its copy number, independent of its position of integration in the mouse genome. Moreover, the expression per gene copy is the same in each mouse and as high as that of the endogenous mouse beta-globin gene. These results indicate that the DNA regions flanking the human beta-globin locus contain dominant regulatory sequences that specify position independent expression and normally activate the complete human multigene beta-globin locus. Further characterization of these control sequences will be presented.

H 211 SUCCESSFUL GENE TRANSFER IN FISH.
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Successful transfer of the neo gene, conferring resistance to the neomycin analog drug G418, into newly fertilized dechorionated goldfish eggs has been performed by microinjection. Multiple copies of the gene are demonstrably incorporated into the high molecular weight fraction of fish DNA, i.e., the genomic DNA. RNA dot blots indicate specific neo mRNA synthesis. Transfer efforts are proceeding using mammalian derived growth hormone genes and the lacZ gene for ß-galactosidase, with a variety of promoters. Phenotypic differences (incresed growth rate) have been noted in putative transgenic goldfish resulting from injection of bovine growth hormone constructs. Current work also includes isolation of piscine promoters and genes for peptide hormones.

This work funded in part by the Minnesota Sea Grant R/A 3 to K.S.G., and the Legislative Commission for Minnesota Resources to A.R.K., P.B.H., A.F., and K.S.G.

H 212 INDUCIBLE CELL SPECIFIC DESTRUCTION IN TRANSGENIC MICE BY EXPRESSION OF HERPES THYMIDINE KINASE,
Richard Heyman, Emiliana Borrelli, Deborah Anderson, Mary Hsi, Jayne Lesley, Robert Hyman and Ronald Evans, Salk Institute, La Jolla, CA 92037.
A technique has been developed in which the selective production of a toxic metabolite in cells expressing herpes thymidine kinase (HSV1-TK) provides a means of killing specific cell types for studies in vitro and in vivo. This selective destruction is achieved by linking the HSV1-TK gene to a cell specific enhancer/promoter; expression of this chimeric gene by itself is innocuous, however, in replicating cells that express HSV1-TK, administration of specific nucleoside analogs, such as acyclovir or ganciclovir (GCV) will inhibit DNA replication leading to cell death. In vitro, the ability of these analogs to inhibit replication is directly proportional to the enzymatic activity of HSV1-TK. Transgenic mice containing the immunoglobulin kappa light chain promoter and the heavy chain enhancer fused to HVS1-TK were generated. Founder lines express varying levels of HSV1-TK activity in spleen, thymus and bone marrow and were apparently normal. Treatment of transgenic animals with GCV for 7 days produces a selective destruction of cells that express HSV1-TK, whereas, this treatment is not toxic to cells that do not express HSV1-TK nor does GCV administration produce toxicity in nontransgenic animals. The cell-specific destruction is a function of the drug concentration, the duration of the treatment as well as the HSV1-TK enzyme activity. This approach will provide a means to selectively destroy specific cells types in an inducible manner thereby allowing studies of mammalian development and cell function.

H 213 IDENTIFICATION OF SPECIFIC GENE SEQUENCES IN PREIMPLANTATION EMBRYOS--DETECTION OF A TRANSGENOME,
Donna King and Robert J. Wall, Reproduction Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705.
Endogenous and foreign DNA sequences can be detected in an extremely small sampling of cells via sequence amplification in vitro. The polymerase chain reaction (PCR) technique applied in multiple cycles allows the amplification of specific short regions of the genome to levels that can be detected by traditional Southern blotting techniques. Cow and mouse blastocysts were analyzed by PCR for the presence of an endogenous single-copy gene or an integrated foreign gene. The single-copy gene encoding the beta chain of bovine luteinizing hormone was detectable in cow blastocysts and in purified bovine genomic DNA representing as few as 15 cells. To determine whether exogenous genes (transgenes) can be detected in preimplantation embryos, transgenic male mice carrying the prokaryotic gene encoding neomycin resistance were bred to nontransgenic females and the resulting blastocysts were analyzed. The neo gene was detected in approximately half of the embryos. The capability to identify specific gene sequences in a limited number of cells allows the further investigation of genetics in early embryos and the recombinatorial mechanisms by which transgenic animals are created.

H 214 INJECTION OF MITOCHONDRIA INTO HUMAN CELLS LEADS TO A RAPID REPLACEMENT OF THE ENDOGENOUS MITOCHONDRIAL DNA, Michael P. King and Giuseppe Attardi, California Institute of Technology, Pasadena, CA 91125.

As an approach to understanding the molecular genetics of human mitochondria, we have developed a procedure to introduce intact mitochondria into human cells. Mitochondria from the CAP^R cell line CAP23 were isolated and injected into the sensitive cell lines 143B and HT1080. The recipient cells had been partially depleted of their mtDNA by pretreatment for several days with ethidium bromide, an inhibitor of mtDNA replication. CAP^R cells were then selected. The conclusion that the CAP^R derivatives of 143B and HT1080 thus isolated represent true mitochondrial transformants is based on four lines of evidence: 1) all the transformants exhibited the same CAP^R mutation of mtDNA as CAP23; 2) the totality of the mtDNA of the transformants had restriction site markers characteristic of the donor cells, while the totality of nuclear DNA in the same cells exhibited an RFLP marker characteristic of the recipient cells; 3) the transformants appeared with a frequency at least 10^4 higher than CAP^R variants in non-injected recipient cell cultures; 4) the drug-resistance phenotype of the transformants was clearly distinct from that of either the donor or the recipient cells. We estimate that, on the average, less than one mitochondrion, representing at most 1-3% of the recipient cell mtDNA complement, was introduced into each injected cell. Yet, at very early times of analysis, several of the HT1080 and 143B transformants contained no detectable CAP^S mtDNA. This complete segregation of the CAP^R mtDNA was achieved in as little as 20-25 generations. These data strongly suggest that intracellular selection of CAP^R mtDNA molecules played a major role in the establishment of these "transmitochondrial" cell lines. Current experiments are focusing on the transfer of mitochondria into cells without the use of a selectable mtDNA marker.

H 215 INAPPROPRIATE EXPRESSION OF THY-1 IN TRANSGENIC MICE CAUSES A LYMPHO-PROLIFERATIVE ABNORMALITY, Carlisle P. Landel, Shizhong Chen, Florence Botteri, Herman van der Putten and Glen A. Evans, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Thy-1 is an abundant cell-surface glycoprotein of unknown function found on mouse T lymphocytes, neurons and hematopoietic stem cells. In order to investigate its function, we re-directed the expression of Thy-1 to the B cell lineage in transgenic mice with a hybrid Thy-1 transgene containing the transcriptional enhancer of the mouse immunoglobulin heavy chain. These mice express the Thy-1.2 transgene on mature B cells and their progenitors and develop a heritable lymphoid hyperplasia characterized by the appearance of several cell populations expressing large amounts of Thy-1 in the bone marrow and lymph nodes. These abnormal cells include a population of large cells expressing both Thy-1 and the pre-B cell antigen B220, and a population of rapidly dividing cells expressing large amounts of Thy-1 but lacking most other cell-surface markers characteristic of the hematopoietic lineage. These mice suffer no grossly detectable immune defects, although we are searching for more subtle effects. We are investigating the function and the developmental potential of these abnormal cells *in vitro* and by transplantation to lethally irradiated mice.

H 216 INTRODUCTION OF THE p53 NUCLEAR ONCOGENE INTO THE MOUSE GERMLINE. Alain Lavigueur[1], Claire Brady[1], Susan Clapoff[1], Victor Maltby[1], David Mock[2], Janet Rossant[1], Sam Benchimol[3], Tony Pawson[1] and Alan Bernstein[1]. [1] Mount Sinai Hospital Research Institute, Toronto, [2] Faculty of Dentistry, University of Toronto, [3] Ontario Cancer Institute, Toronto, Canada.

In order to study the biological consequences of the overexpression of the oncogene p53, we have generated transgenic mice by DNA microinjection. A 15 Kb EcoRI-Bgl II p53 genomic fragment isolated from the mouse erythroleukemia cell line CB7 was used in these studies. Western blot analysis of four different transgenic mice lines showed that the level of the p53 gene product was very high in spleen, thymus, lymph nodes and ovaries whereas lower but significant levels were observed in brain, liver, kidney, lung, skeletal muscle and testis when compared to control animals. We have observed tumor formation including sarcomas and carcinomas in animals from 4 to 10 months old. We have also investigated the effects of p53 overexpression on progenitors cells within the hematopoietic system. The results demonstrated a significant increase in the numbers of erythroid progenitor cells (BFUe) in p53 transgenic mice whereas the numbers of granulocyte-macrophage precursors (CFU-GM) remain unchanged.

This work is supported by the NCI and the MRC of Canada.

H 217 REGULATION OF RAT β-CASEIN GENE EXPRESSION IN TRANSGENIC MICE, Kuo-Fen Lee, Francisco J. DeMayo, Suzanne H. Atiee and Jeffrey M. Rosen, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
The rat β-casein gene is a member of a small gene family. To elucidate the molecular mechanisms by which its expression exhibits tissue- and stage-specific as well as hormonal regulation, a 14-kb genomic clone containing the entire rat β-casein gene plus 3.5 kb of 5' and 3.0 kb of 3' flanking DNA sequences was introduced into mouse embryos. A specific RNase protection assay demonstrated that the transgene was initiated at the authentic transcription start site, and was expressed predominantly in the lactating mammary gland of transgenic mice at a level of 0.01-1% of the endogenous mouse β-casein gene. To further define the minimal DNA sequences required for tissue-specific expression, 2 casein CAT (chloramphenicol acetyltransferase) fusion genes were introduced into mouse embryos separately. CAT activity was detected predominantly in the lactating mammary gland of transgenic mice bearing either fusion gene, demonstrating that 0.5 kb of 5' flanking DNA sequences, exon 1 and part of intron A are sufficient to direct the CAT gene expression selectively in the mammary gland. Moreover, the transgenes appeared to be developmentally regulated in accordance with the endogenous gene. Addition of prolactin to mammary explants from transgenic mice bearing the fusion genes in a medium containing insulin and hydrocortisone resulted in a 31-fold induction of the endogenous gene, but only a 5-fold induction of CAT activity, suggesting that although the casein promoter region may be sufficient to confer tissue-specific expression, other sequences either within or 3' to the gene may be required to elicit hormonal regulation. Mammary explant cultures from mice containing the entire rat β-casein gene are in progress to establish this definitively. Supported by USDA grant 86-CRCR-1-2250.

H 218 DIPHTHERIA TOXIN A-CHAIN EXPRESSION DIRECTED TO B-LYMPHOID CELLS, Ian H. Maxwell, L. Michael Glode and Françoise Maxwell, University of Colorado Health Sciences Center, Denver, CO 80262
It has previously been demonstrated that targeted cell killing can be achieved using DNA constructs in which the coding sequence for the A-fragment of diphtheria toxin (DT-A) is linked with appropriate transcriptional regulatory elements (Maxwell *et al.* Cancer Res. 46 4660 (1986); Palmiter *et al.* Cell 50 435 (1987); Breitman *et al.* Science, in press). To target B-lymphoid cells, we have constructed DT-A expression plasmids containing immunoglobulin (Ig) enhancers and promoters. Toxicity was assessed using a transient co-transfection assay in which the DT-A test construct was electroporated into cultured cells together with a luciferase-expressing reporter plasmid. Inhibition of protein synthesis by DT-A expression resulted in greatly diminished luciferase activity in extracts of the transfected cells. The construct pTH17, containing human H chain enhancers, efficiently expressed DT-A (from a mouse V_H promoter) in murine and human B-cells but not in HeLa. We shall present the results of experiments to assess DT-A expression from plasmids containing various combinations of Ig enhancers and promoters in cultured B- and T-lymphoid cells and other cell types.

H 219 PATHOLOGICAL LESIONS IN THE LIVER OF METALLOTHIONEIN-OVINE GROWTH HORMONE TRANSGENIC MICE, J.M. Orian, C.S. Lee and M.R. Brandon, The University of Melbourne, Australia.
Transgenic mice containing a metallothionein 1-ovine growth hormone fusion gene were produced and the effects of chronic exposure to high levels of circulating ovine growth hormone (oGH) analysed in three generations of mice. In contrast to results reported for female mice expressing human, rat and bovine growth hormones, mice expressing oGH were fertile and their pituitaries appeared functional. Livers of transgenic mice expressing ovine growth hormone were enlarged irrespective of the level of serum oGH. In mice expressing high levels of oGH, direct measurements of hepatocytes in liver sections revealed that cell and nuclear sizes were abnormally large. Hepatocytes in different transgenic mice varied from 1.4 to 2.2 times normal size and hepatocyte nuclei varied from 1.65 to 2.4 times normal size. In addition, intranuclear inclusions were observed in hepatocytes of transgenic mice, and these inclusions appeared to be produced by invaginations of the nuclear envelope containing cytoplasmic material. Their presence was always associated with high levels of serum growth hormone. The above observations have relevance to studies aimed at correcting human genetic diseases by gene transfer.

H 220 ONCOGENE-INDUCED T CELL TUMOURS EXPRESSING A FOREIGN PROTEIN IN TRANSGENIC MICE,

A. Pavirani, T. Skern, M. Le Meur*, D. Ali-Hadji, A. Benavente, B.Bouderbala, P. Gerlinger* and Courtney M. Transgene S.A., 11, rue de Molsheim and *Institut de Chimie Biologique, 11, rue Humann, 67000 Strasbourg, France.

Multicentric lymphoid tumours were generated at high frequency in transgenic mice carrying DNA constructions with either mouse c-myc or SV40 virus T-antigen genes and a cDNA encoding the Pittsburgh variant of human α_1-antitrypsin, α_1-AT(Arg^{358}), a potent thrombin inhibitor. The oncogene and α_1-AT(Arg^{358}) sequences were inserted downstream from the mouse IgH and IgK promoters respectively and the transcription units were placed in divergent juxtaposition with a copy of the IgH enhancer located centrally. Transgenic mice carrying tumours expressed human α_1-AT antigen in the serum at ~20 µg/ml. When propagated by intraperitoneal transplantation in syngeneic mice the tumours secreted the foreign protein leading to the presence of circulating α_1-AT(Pittsburgh). Furthermore the c-myc derived tumour cells were cultured in vitro and these secreted α_1-AT(Arg^{358}) for more than three months in culture. Fluorescence Activated Cell Sorter analysis indicated that both c-myc and T-antigen tumours were T cell in origin, being 100 % Thy-1 positive and expressing no surface immunoglobulin. Moreover, it was shown that the c-myc tumours express the c-myc transgene but not endogenous c-myc whereas the T-antigen tumours express both T-antigen and endogenous c-myc. We are currently evaluating the potential of this approach for the isolation of stable cell lines producing foreign proteins.

H 221 CHIMAERIC FISH ANTIFREEZE PROTEIN GENES IN TRANSGENIC DROSOPHILA,

Derrick E. Rancourt, V.K. Walker* and P.L. Davies. Departments of Biochemistry and Biology*, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

To enhance survival in sub-zero temperatures, select groups of marine fish and terrestrial insects produce serum antifreeze proteins (AFP) which act non-colligatively to depress the freezing point of their body fluids. The winter flounder (Pseudopleuronectes americanus) uses a set of alanine-rich, alpha-helical AFP's, which are encoded in a multigene family whose individual members are 1kb long and contain a single intervening sequence. We are interested in conferring limited freeze resistance to other organisms by AFP gene transfer and have initiated gene transfer programs using commercially important fish and plants. In addition, as a model system we have used P element vectors to transfer AFP gene chimaeras to the Drosophila germ line. Transformed lines containing an hsp70-AFP fusion gene show two heat shock-induced AFP transcripts that are correctly spliced, polyadenylated and translated to yield AFP in Drosophila hemolymph. To increase AFP levels in hemolymph we are presently using AFP gene constructions regulated by the Drosophila yolk protein (YP1) promoter.

H 222 EXPRESSION OF A MYELIN BASIC PROTEIN GENE IN TRANSGENIC SHIVERER MICE: CORRECTION OF THE DYSMYELINATING PHENOTYPE

Carol Readhead, Brian Popko, Naoki Takahashi, H. David Shine, Raul Saavedra, Richard L. Sidman and Leroy Hood, California Institute of Technology, Pasadena, CA 91125 and Harvard Medical School, Boston, MA 02115.

Mice homozygous for the autosomal recessive mutation shiverer (shi) lack myelin basic protein (MBP). The central nervous system of these mutant mice is hypomyelinated and myelin, where it exists, is uncompacted. Shiverer mice exhibit a distinct behavioral pattern including tremors, convulsions and early death. We have previously demonstrated that shiverer mice have a partial deletion of the gene encoding MBP. Now we are able to demonstrate that the abnormal myelination and shivering phenotype are due to a single gene defect. This was achieved by introduction of the wild-type MBP gene into the germline of shiverer mice by microinjection into fertilized eggs. Shiverer mice homozygous for the transgene have MBP mRNA and protein levels that are approximately 25% of normal. The expression of the MBP transgene is tissue specific and developmentally regulated. Moreover, the four different forms of MBP produced by alternative patterns of RNA splicing are present. These mice have compacted myelin with major dense lines though the myelin sheaths are thinner than normal. Transgenic shiverer mice homozygous for the MBP transgene no longer shiver nor die prematurely.

H 223 CHICKENS TRANSGENIC FOR A DEFECTIVE RECOMBINANT AVIAN LEUKOSIS PROVIRAL INSERT EXPRESS SUBGROUP A ENVELOPE GLYCOPROTEIN AND ARE HIGHLY RESISTANT TO A PATHOGENIC SUBGROUP A AVIAN LEUKOSIS VIRUS, D.W. Salter, Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824 and L.B. Crittenden, USDA-ARS Regional Poultry Research Laboratory, East Lansing, MI 48823.

We have produced a large number of chickens transgenic for recombinant avian leukosis virus (ALV) proviral DNA. Most of them inherited proviral inserts that coded for complete infectious ALV. One of the inserts was defective for infectious ALV production and did not produce ALV group-specific antigen. We backcrossed males heterozygous for this insert with Line 0 females. Chicken embryo fibroblasts from embryos carrying this proviral insert expressed subgroup A envelope glycoprotein in a chick-helper-factor assay and were about 5000-fold more resistant to subgroup A sarcoma virus than those lacking the insert. Chicken embryo fibroblasts with or without the insert were equally susceptible to subgroup B sarcoma virus. In a similar mating, progeny chicks with or without the insert were inoculated at day of hatch with a field strain of subgroup A ALV. Viremia, antibody, and ALV-specific mortality were determined through 40 weeks of age. Insert-containing chickens were negative for subgroup A ALV-induced viremia, antibody and mortality. Chickens lacking the insert were highly viremic, produced antibody and many have died of ALV-specific neoplasms. This defective recombinant ALV proviral insert represents an artificially introduced dominant gene for resistance to subgroup A leukosis-sarcoma viruses that cause loss of productivity and mortality in commercial chickens.

H 224 TARGETED INTRODUCTION AND EXPRESSION OF ACTIVATED GENES INTO POST-IMPLANTATION RAT EMBRYOS VIA INJECTION OF TRANSFECTED RAT EMBRYO CELLS, Stephen A. Schwartz, Ross Couwenhoven, and Stanley Welch, The Chicago Medical School, North Chicago, Ill. 60064
The generation of transgenic animals has become a valuable tool to investigate molecular mechanisms for regulation of cell growth, division, and differentiation. Retroviral vectors and micro-injection have been conventionally used to introduce genes into mammalian embryos. Due to the randomness and non-specificity of these methods, however, it is difficult to generate transgenic or mosaic animals with respect to a particular germ line derivation. We developed a technique to simply and reproducibly generate mosaic rats containing exogenous genes exclusively in tissues of mesenchymal derivation. Primary cultures of 16 day old rat embryo fibroblasts were transfected with the pSV2neo gene in an expression vector, selected with G-418, and injected into 9-10 day old rat embryos. Inasmuch as the fibroblasts are totipotent mesenchymal precursors, the rat offspring are genetic mosaics in cells and tissues of mesenchymal origin. Nearly 60% of the injected animals survived to birth, of which nearly 50% contained and expressed pSV2neo as determined by Northern and Southern blotting. The tissues containing neo were primarily confined to spleen, bone marrow, cardiac and skeletal muscle, and stroma from major visceral organs. When the activated human Ha-ras-1 was similarly introduced into rat embryos, nearly all of the mesenchymal-derived tissues contained the oncogene, although the surviving offspring are apparently healthy during the first 4 months of observation. Similar studies are underway to introduce other DNAs into epithelial precursor cells in order to generate mosaic rats with exogenous genes exclusively in tissues of epithelial derivation.

H 225 HPV-1 AND EBV GENES IN TRANSGENIC MICE, Peter F. Searle and Jonathon M. Tinsley, University of Birmingham, Birmingham B15 2TJ, England.
Human papillomavirus type 1 (HPV-1) infection of man causes the production of benign palmar and plantar warts. As yet there is no suitable tissue-culture system to study HPV-1 infection. We have inserted the HPV-1 genome into transgenic mice, but expression was undetectable. No tumours have been produced. We have now made constructs in which the HPV-1 early region is placed under the control of regulatory sequences from keratin genes, and are inserting these into mice.

Epstein-Barr virus (EBV) infection of man is widespread and usually asymptomatic. However in certain regions of the world EBV has been implicated in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma. In vitro EBV immortalises B-lymphocytes, and expresses a restricted number of genes during this latent infection. We are attempting to express the EBV latent gene products in transgenic mice to test their possible function in tumourigenesis.

H 226 Expression of Active Human Antihemophilic Factor IX in the Milk of Transgenic Sheep.

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During lactation, large amounts of protein are synthesized by the mammary gland and secreted into the milk. We have previously shown the expression of sheep β-lactoglobulin (BLG) in the mammary glands of transgenic mice, and it's secretion into the milk at high concentration, up to 5 times that found in sheep milk (Nature 328, 530). Following along this line, we are investigating the potential of transgenic animals for the production of large amounts of authentic human proteins in milk.

Patients with hemophilia B are deficient in clotting Factor IX (FIX), and are currently treated with a FIX concentrate prepared from human plasma. To direct expression of FIX to the mammary gland of transgenic animals, we have construced a BLG-FIX fusion gene. Microinjection of DNA into fertilized sheep eggs was used to produce transgenic sheep carrying the BLG-FIX gene. Four transgenic sheep were obtained, two male and two female. The two female sheep were bred, and milk collected. Human FIX was detected by radioimmunoassay, and after partial purification was shown to be active in a clotting assay. Northern analysis of RNA from biopsies of mammary tissue show that the BLG-FIX fusion gene is expressed in the mammary gland. Three of the transgenic sheep have transmitted the BLG-FIX fusion gene to their progeny.

H 227 GERM-LINE TRANSFORMATION OF THE ZEBRAFISH, BRACHYDANIO RERIO

Gary Stuart, James McMurray, and Monte Westerfield. Institute of Neuroscience, University of Oregon, Eugene, OR 97403

In an effort to generate stable lines of transgenic fish, early zebrafish embryos were injected with high concentrations of foreign DNA. After injection, a linearized bacterial plasmid was converted into a high molecular weight form and then amplified approximately ten fold during the initial rapid cleavages characteristic of the early blastula. While most of this DNA was subsequently degraded during gastrulation, some of the foreign sequences survived the gastrula stage and could be found in greater than 90% of injected fish at 3 weeks of age. However, only about 5% of these fish contained foreign sequences in their fins at 4 months, and less than 0.5% contained foreign DNA in the germ-line. While only 20% of the F_1 offspring derived from one germ-line positive parent actually inherited the foreign DNA, this DNA could be found in approximately 50% of the F_2 progeny derived from identified F_1 fish. These observations suggest that injected DNA can be integrated into the fish genome and that the resulting transgenic fish are mosaic. We wish eventually to adapt our fish transformation system to the study of developmental gene expression and the analysis of mutations caused by the insertion of foreign DNA.

H 228 PITUITARY-SPECIFIC EXPRESSION OF SV40 T-ANTIGEN IN TRANSGENIC MICE,

Jolene Windle, Richard Weiner*, and Pamela Mellon, The Salk Institute, La Jolla, CA 92037 and *University of California, San Francisco, CA 94143.

We have generated transgenic mice in which expression of an oncogene is directed to specific cell types of the anterior pituitary. The SV40 T-antigen (Tag) gene has been placed under the control of two different pituitary-specific promoters, those of the human glycoprotein hormone α-subunit and luteinizing hormone (LH) β-subunit genes. The α-subunit is common to LH, follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH), and thus its promoter should direct expression to both pituitary gonadotropes and thyrotropes. In contrast, the β-subunit is unique to LH, and therefore is expressed only in gonadotropes.

Eight founder mice containing the α-Tag hybrid gene have been obtained. Four of these, three females and one male, developed large pituitary tumors by three months of age. Each of these tumors was shown to be expressing a high level of T-antigen mRNA, but levels of α-subunit mRNA varied widely among the different tumors.

In addition, 12 lines of mice containing the β-Tag hybrid gene have been obtained. While none of these mice have developed tumors, we have observed hyperplasia and increased vascularization of the pituitary in several lines. Low levels of T-antigen mRNA have been detected in pituitaries of mice from several of the lines, with the level gradually increasing with age. The fragment of β-subunit promoter used in this hybrid gene (from -1000 to +9) apparently does not contain the sequences mediating steroid regulation of the this gene, since castration of these mice does not result in elevation of T-antigen mRNA.

H 229 DEVELOPMENTAL REGULATION OF A HOMEOBOX GENE IN TRANSGENIC MICE, Jozsef Zakany, Mayuri Patel, Christopher Tuggle and Chi Nguyen-Huu, Columbia University, New York, 10032.
In search for genes wich may be regulating mammalian development the homeobox containing genes are found to be the most likely candidates. Several mouse homeobox genes are expressed in specific regions of the midgestational stage embryo. We have isolated and characterized a murine homeobox gene localized on chromosome 6 designated Hox-1.3. Studies of 11.5 days old embryonic RNA samples show that the gene is expressed at high level in the spinal cord, not at all in the brain, while a lower level is detected in the rest of the embryo proper.
Transgenic lines were generated containing the Hox-1.3 gene with the E. coli lacZ structural gene inserted immediately downstream of the initiation codon. Four of seven tested transgenic lines express histochemically detectable β-galactosidaze in the embryonic spinal cord, the expression is undetectable in the forebrain anlage and several other body regions outside the central nervous system also express the transgene. Although the detailed description of the expression pattern is still in progress we conclude that the 4.3 kb mouse component of the transgene harbours the cis-acting elements which mediate developmental regulation of the Hox-1.3 gene in a variety of embryonic cells.

Recombination, Enhancers and Genetic Diseases

H 300 INTEGRATED ROUS SARCOMA PROVIRUSES AS PROBES TO STUDY GENE EXPRESSION IN MAMMALIAN CELLS. Jane E Akroyd, Tony R Green and John A Wyke. Imperial Cancer Research Fund Dominion House, St Bartholomew's Hospital, Bartholomew Close, London, EC1, UK.
The Rous sarcoma provirus is a self-sufficient genetic entity, bearing in its flanking LTR's sequence elements that promote and enhance transcription of viral genes, including the v-src oncogene. Although these regulatory elements act as a potent promoter and enhancer in transient expression assays in rat cells their effect is usually silenced when the provirus integrates in rat cellular DNA and proviral transcription is not detectable. This effect is not due to the presence of inhibitory cellular factors that are titrated out in the transient assays and the rare transforming events are not the result of viral or cell mutations (Akroyd et al, 1987). Our working hypothesis is that proviral expression is determined by differential cell-regulation of transcription at various integration sites in the cell genome. Recent DNA transfection experiments have shown that cellular sequences near the integration site can regulate proviral expression in cis (Akroyd et al, 1987)and where examined changes affecting the chromatin structure and activity of the provirus extend into the flanking cell DNA. Regulation of proviral expression can also be mediated in trans when actively expressed proviruses integrated at certain sites can be silenced by cell fusion to a normal cell. The aim of our work is to identify and determine the mode of action of these regulatory elements. The 5′ cellular sequences flanking both expressed and transcriptioanlly silent proviruses have been cloned, and experiments are in progress to isolate the "silencer" sequences for further analysis.
Akroyd et al, 1987. Oncogene: in press

H 301 INHERITED DISORDERS OF KERATINIZATION: MUTATIONS IN A HUMAN KERATIN GENE. M. Blumenberg, E.S. Savtchenko and I.M. Freedberg, Departments of Dermatology and Biochemistry, NYU Medical Center, New York, NY 10016.
A number of human inherited keratinization disorders exhibit altered expression of epidermal keratin genes. Because of their accessibility, keratinocytes are an excellent candidate for vectorin somatic gene therapy and recently a system has been developed for DNA delivery into these cells (Morgan et al., 1987, Sci. 237;1476).
At least 20 different keratin proteins have been described in different human epithelia. We have determined the complete DNA sequence of a human keratin gene. Comparison of our data with published sequences indicates that the gene we have sequenced is a product of a very recent gene duplication of the K14 acidic type keratin gene. The two sequences show 95% identity which extends into the untranslated regions. This high homology compares with 70-80% similarities among keratin genes belonging to the same subfamily.
Our sequence does not represent the functional gene, however, because of a number of mutations which render it inactive. The most striking one is a small, 8-base pair duplication in the third exon. Three mutations destroy RNA processing signals, two are in intron/exon boundaries and one disrupts the polyadenylation signal.
The regulatory region of this gene is virtually identical to the functional K14 gene counterpart. We therefore believe that this pseudogene is expressed with the same regulatory mechanism as the functional gene. Since the functional gene is expressed in the basal layer of stratified epithelia, the regulatory region of our gene is potentially an excellent vehicle for introducing and targeting genes into the basal keratinocytes.

H 302 ANALYSIS OF EUKARYOTIC GENE EXPRESSION *IN SITU* USING A REPORTER GENE ACTIVATED BY CHROMOSOMALLY-LOCATED TRANSCRIPTIONAL AND TRANSLATIONAL SIGNALS, Daniel G. Brenner and Stanley N. Cohen, Stanford University, Stanford, CA 94305.
In order to identify and study the expression of developmentally-regulated genes, a series of vectors (SIN/*lac*) has been constructed to allow activation of a reporter gene carried by a retrovirus upon integration of the virus into an expressed region of the chromosome. As in the case of the Mu-*lac* constructs created to investigate gene expression in prokaryotes, the retroviral-based system is designed to fuse the *lac* gene to chromosomally-located promoters, thus serving as an *in vivo* probe for regulatory sequences. The choice of the *E. coli lacZ* gene as the reporter also allows the formation of enzymatically-active *in vivo* fusions of β-galactosidase (β-gal) with eukaryotic proteins, and hence the analysis of translational, as well as transcriptional, control. Self-inactivating (SIN) retroviruses that lack both the enhancer and the promoter of the LTR have been employed as vectors to allow maximal expression of the reporter gene. The recent development of a technique to sort live cells expressing β-gal using a fluorescence-activated cell sorter (G. Nolan and L. Herzenberg, personal communication) has increased the sensitivity of detection and has made practical the isolation of clones that express β-gal. The expression of β-gal activity in cos-7 cells infected transiently by SIN/*lac* constructs carrying a known promoter adjacent to the disabled retroviral LTR has demonstrated the validity of the approach. Additionally, co-cultivation of SIN/*lac*-producing Psi-2 cells together with mouse lymphoblastoid cells has resulted in the activation of β-gal expression in the lymphoblastoid cells. Experiments currently are in progress to investigate the utility of the SIN/*lac* system by analysis of the activity of the *hgprt* promoter using the selectable insertional inactivation of the *hgprt* locus in a male cell line.

H 303 HUMAN RECOMBINANT ADENOVIRUS USED TO CORRECT A MOUSE ENZYME DEFICIENCY.
Jean-Francois Chasse*, Michel Perricaudet#, Michele Minet-Thurriaux*, Pascale Briand*, Massimo Levrero#, *LBG Necker Hospital Paris, and #IRSC Villejuif, France.
Human ornithine transcarbamylase deficiency is one of the most frequent hereditary hyperammoneamia. This X-linked inherited disease leads to the death of 75% of the affected males, watever the treament. Thus although several types of mutations leading to various levels of residual activity have been described, the evolution 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 last injection. Concomitantly to the expression of the introduced OTC gene, a corrected phenotype could be observed in some animals.

H 304 COMPLEMENTATION OF THE UV SENSITIVE PHENOTYPE OF A XERODERMA PIGMENTOSUM HUMAN CELL LINE BY TRANSFECTION WITH A cDNA CLONE LIBRARY,
Dan Canaani, Tal Teitz and Tova Naiman, Department of Biochemistry, Tel Aviv University, Tel Aviv 69978, Israel.
We report the complementation by gene transfer of the UV sensitivity of an XP-C established cell line. A human cDNA clone library constructed in a mammalian expression vector, and itself incorporated in a lambda phage vector, was introduced into the cells as a calcium phosphate precipitate. Following selection to G418 resistance, transformants were selected for UV resistance. Twenty-one cell clones were obtained with UV resistance levels typical of normal human fibroblasts. Upon further propagation in the absence of selection for G418 resistance, about half of the primary transformants remained UV resistant. Secondary transformants were generated by transfection with a partial digest of total chromosomal DNA from one of these stable transformants. This resulted in G418 resistant clones, several of which exhibited UV resistant phenotype. The other primary clones lost UV resistance rapidly when subcultured in the absence of G418. Importantly, several retained UV resistance under G418 selection pressure. The acquisition of UV resistance by secondary transformants derived by transduction of DNA from a former clone, and the linkage between G418 and UV resistances in the second group, strongly suggests that the XP-C transformants acquired UV resistance through DNA-mediated gene transfer and not by reversion.

H 305 CLONING AND ANALYSIS OF HUMAN PHENYLALANINE HYDROXYLASE, DIHYDROPTERIDINE REDUCTASE AND PYRUVATE DEHYDROGENASE.
H.-H.M. DAHL, R.G.H. COTTON, G.K. BROWN AND D.M. DANKS, MURDOCH INSTITUTE, ROYAL CHILDREN'S HOSPITAL, MELBOURNE, AUSTRALIA, 3052. Our study of inborn errors of metabolism focuses on hyperphenylalaninemia and lactic acidosis. We have isolated full-length human cDNA clones for phenylalanine hydroxylase (classical phenylketonuria), dihydropteridine reductase (malignant hyperphenylalaninemia) and pyruvate dehydrogenase E1α subunit (primary lactic acidosis). The DNA sequence of these clones has been determined, and RFLPs useful in prenatal diagnosis have been found. Analysis of patient cell lines and biopsies by Southern, Northern and Western blotting has shown that the mutations causing the defects in all three diseases are heterogeneous. Neurological disturbances are often seen in malignant hyperphenylalaninemia and lactic acidosis, and the disorders are lethal in their severe forms. Furthermore, no satisfactory treatments are available. At present we are therefore studying the regulation of these genes by analysing the effects of various metabolites and hormones on gene expression. Tissue specific regulation is also being investigated. To facilitate these studies we have isolated genomic clones and are in the process of characterising the promotor regions. Understanding the regulation of these enzymes is important if the DNAs is to be used in gene transfer experiments. Although there will be significant problems of targeting the gene products to all relevant cells, these genes are obvious candidates for use in gene therapy.

H 306 GENE TARGETTING WITH RETROVIRAL VECTORS. James Ellis*, Raju Kucherlapati#, and Alan Bernstein*, Mt. Sinai Hospital Research Institute, Toronto, Ontario Canada*, and Center for Genetics, University of Illinois, College of Medicine, Chicago, Illinois#.

We have designed a model system to investigate whether retroviral vectors can target exogenous genes into specific chromosomal loci. Retroviral vectors have been constructed that have a 2bp deletion within the att region of U_5 to prevent efficient integration by the normal retroviral mechanism. Into this backbone a nonfunctional neo gene with a large deletion in the 3' coding region was inserted. As assayed by an RNA dot blot procedure, this vector (MoDR) produces virus particles at high titer ($1X10^6$) after transfection into PA317 packaging cells. When the virus is used to infect human cells bearing a nonoverlapping neo gene deletion, targetted integration of the virus should reconstruct a functional neo gene that can be directly selected for with the drug G418. An example of successful targetting will be presented. Experiments are currently in progress to determine the frequency and mechanism of gene targetting with retroviral vectors. (This work is supported by grants from the MRC and the NSERC of Canada).

H 307 TISSUE SPECIFIC INDUCIBILITY OF BETA-INTERFERON. John F. Engelhardt and Paula M. Pitha, The Johns Hopkins University Oncology Center, Baltimore, Maryland 21205.

The effects of the tissue specific immunoglobulin heavy chain enhancer (Igenh) on the inducible transcription of human beta-interferon (human beta-IFN) was analyzed. To facilitate efficient gene transfer in various cell types, a modified pLJ retroviral vector was used. The 3' LTR viral enhancer was deleted from pLJ (pLJenh-) to minimize complications from full-length viral transcription and possible regulatory effects on beta-interferon expression. Vector constructs including the Igenh and human beta-IFN gene segments, were designed in various orientations and combinations in both pLJenh- and pLJenh+ vectors. Following packaging in psi-2 cells, these constructs were tested by infecting NIH/3T3 and A20 (mouse B-cells) followed by selection in G418. Newcastle Disease virus (NDV) infection was employed as the method of inducing both endogenous mouse beta-IFN and exogenous human beta-IFN. Following NDV induction, Northern analysis was used to compare the efficiency of human beta-IFN transcription within the various constructs while using the level of endogenously induced beta-IFN as an internal control.

H 308 REGULATION OF VISNA VIRUS, Dana H. Gabuzda, Jay Hess, and Janice E.Clements, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Visna virus, a member of the lentivirus subfamily of retroviruses, causes chronic pneumonitis and inflammatory neurologic disease in sheep. The latent infection of monocytes is a characteristic feature of visna virus and other lentiviruses, including the Human Immunodeficiency Virus. Viral gene expression is highly restricted in monocytes but is activated during monocyte differentiation into macrophages. We are investigating the developmental regulation of visna virus transcription in U937 cells by transfecting plasmids containing the visna long terminal repeat (LTR) directing bacterial chloramphenicol acetyltransferase gene transcription and assaying transient expression. In addition, we are analyzing a series of deletion and linker-scanner mutations to identify regions of the visna LTR involved in the regulation of transcription. Visna LTR expression is induced by phorbol 12-myristate 13-acetate, a phorbol ester which induces macrophage-like differentiation. The identification of viral and cellular factors which regulate visna viral gene expression in vitro may provide insight into molecular mechanisms involved in the pathogenesis of diseases caused by visna and other lentiviruses.

H 309 REGULATED EXPRESSION OF TRANSFECTED DIPHTHERIA TOXIN A CHAIN GENE TO ELIMINATE TARGET CELL POPULATIONS. L. Michael Glode, Ian H. Maxwell, Francoise Maxwell, University of Colorado Health Sciences Center, Denver, Colorado 80262 and Ismo Ulmanan and Arja Kallio, Helsinki, Finland.

As an alternative to the use of antiproliferative drugs or monoclonal antibody-targeted toxins, we have shown that specific cell populations can be induced to "commit suicide" by the introduction of DNA coding for the diphtheria toxin A-chain (DT-A) and containing appropriate transcriptional regulatory signals (Cancer Res. 46 4660 (1986); Mol. Cell. Biol. 7 1576 (1987)). That exquisite specificity is possible with this approach has been demonstrated in collaborative efforts in which exocrine pancreatic or lens cells were specifically eliminated in transgenic mice (Palmiter et al. Cell, in press; Breitman et al. submitted). We are now constructing vectors to enable inducible, as opposed to developmental, triggering of cell suicide. In preliminary experiments we have expressed the DT-A gene from a human heat shock gene (hsp70) promoter contained in a vector including EBV ori-P and EBNA-1 DNA (intended to prevent integration and possible rearrangement of the construct). It was found that stable transformants could be selected when an integrating vector was used, whereas none were obtained from the non-integrating construct. Further experiments using the heat-shock promoter or the IL-2 promoter to regulate toxin gene expression will be presented.

H 310 IDENTIFICATION OF AN ANDROGEN-REGULATED ENHANCER SEQUENCE AND A TISSUE-SPECIFIC CIS DNA ELEMENT ENHANCER SEQUENCE IN THE RAT SEMINAL VESICLE IV GENE, Stephen E. Harris, Marie A. Harris and R.G. Smith, W. Alton Jones Cell Science Center, Lake Placid, NY 12946, and Merck, Sharpe and Dome, Rawway, NJ.

The rat seminal vesicle produces a set of androgen-responsive secretory proteins, SVS I-VI. We have mapped an androgen response element (enhancer) and a potential tissue-specific cis DNA element in the 5'-flanking region (-270 to -70) of the SVS IV gene by transfection of pSVS IV -523 to -10/CAT construction into primary rat seminal vesicle epithelial cell cultures. To extend these studies, we prepared a large 5'-flanking (-4200 to -10) SVS IV construction ligated to human growth hormone gene (into the Bam H1 site of pØ GH-Allegro). Various 5'-deletions from -4200 to -100) ligated to this pØ GH (GH = growth hormone) were also prepared by exo III/Mung bean unidirectional deletion preparation (Stratagene). These constructions are being transfected into primary rat seminal vesicle epithelial cell cultures, from young 30 day old animals, grown in the presence and absence of 10^{-7} M testosterone. The media is then assayed for the presence of GH using ^{125}I-anti-growth hormone (Allegro). Some of these same SVS-GH constructions are being used to produce transgeneic mice which express the hGH under androgen control in the seminal vesicle or other tissues. Progress on these experiments will be presented.

H 311 ADENOVIRUS VA RNA ENHANCES TRANSLATION BY INHIBITING PHOSPHORYLATION OF THE ALPHA SUBUNIT OF EUKAROTIC INITIATION FACTOR 2 (eIF-2a). Randal J. Kaufman*, Monique V. Davies*, Vinay Pathak', and John Hershey'. *Genetics Institute, Cambridge, MA and the 'Department of Biological Chemistry, University of California School of Medicine, Davis, CA

The translational efficiency of mRNA molecules transcribed from plasmid DNA transfected into COS-1 monkey cells can be increased 10- to 20-fold by the coexpression of the adenovirus virus-associated (VA) RNA I or by the addition of 2-aminopurine, an inhibitor of the double-stranded RNA activated protein kinase, to the medium. Both the effects of the VA genes and 2-aminopurine are specific to the mRNAs derived from the transfected plasmid DNA and do not alter host mRNA translation. The role of eIF-2a phosphorylation has been studied by expression of wildtype and mutant (containing serine to alanine changes at amino acid residues 48 or 51, putative sites of phosphorylation) eIF-2a cDNA genes. Expression of either mutant eIF-2a gene resulted in a similar increase in translation of a cotransfected gene as that obtained by co-expression of VA RNA I or by treatment with 2-aminopurine. In contrast, expression of the wildtype eIF-2a gene resulted in negligible increase in translation of the cotransfected gene. These results demonstrate that inefficient translation of mRNAs derived from plasmid DNA in COS-1 cells results from the phosphorylation of eIF-2a and are consistent with translational control models invoking the requirement for catalytic use of the eIF-2a recycling factor to maintain efficient mRNA translation of an mRNA. The eIF-2a genes have also been stably introduced into human 293 cells. Data will be presented on the ability of these 293 cells to complement adenovirus VA deficient mutants.

H 312 DOMINANT MUTATION IN GALACTOSYLTRANSFERASE CREATED BY OVER-EXPRESSION OF A TRUNCATED cDNA, Vincent J. Kidd, Helen Fillmore and Bruce Bunnell, University of Alabama at Birmingham, Birmingham, AL 35294.

Using a truncated cDNA clone for the glycosyltransferase, β1-4 galactosyltransferase (GalTase), and a vector containing a DHFR mini-gene we have produced a CHO cell line that produces less than 10% of the normal endogenous GalTase gene products. Analysis of GalTase enzyme activity from cells containing increasing amounts of the truncated GalTase mRNA shows proportionately decreasing amounts of endogenous golgi (40 kD) and cell-surface (80 kD) enzyme activities. Likewise, Western blot analysis reveals a similar proportional decrease in both GalTase proteins. This mutation is, however, lethal at high levels of exogenous truncated GalTase mRNA, presumably due to the involvement of cell-surface GalTase in cellular growth. The GalTase mutant cells are not only arrested in growth, but also demonstrate alterations in glycoprotein biosynthesis and changes in the micro-structure of the golgi. This type of genetically engineered cell mutant provides a powerful new technique to look at the cellular functions of isolated gene products.

H 313 ISOLATION AND CHARACTERIZATION OF THE DUCHENNE MUSCULAR DYSTROPHY GENE PROMOTER REGION. H.J. Klamut, A.H.M. Burghes, S. Bodrug, C. Duff, S. Malhotra, P.N. Ray and R.G. Worton, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

Evidence has recently been obtained which indicates that Duchenne muscular dystrophy (DMD) gene expression in human myogenic cultures is dependent on differentiation of myoblasts into multinucleated myotubes. Complimentary DNA clones isolated in our laboratory which correspond to the 5' end of the DMD gene have been used to screen a Sau3a XXXX cosmid library in order to generate genomic clones containing the first 7 exons of the DMD gene. One of the cosmid clones isloated (designated XJc8) has been found to contain the most 5' exon of the DMD region. A 1.4 kb HindIII-PstI fragment containing this exon has been subcloned into pBSm13 for sequence analysis of exon boundaries and potential upstream promoter regions. Sequences having potential for promoter activity will be subcloned initially into pSVOCAT vectors and transfected into myogenic cell lines for analysis of DMD gene promoter activity during the process of myoblast differention. It is anticipated that this study will provide valuable insights into the structure and function of the DMD promoter and the regulation of DMD gene expression.

H 314 DETERMINATION OF MUTATIONS IN FACTOR IX BY GENOMIC AMPLIFICATION WITH DIRECT SEQUENCING, Dwight D. Koeberl and Steve S. Sommer, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905.
Genomic amplification with direct sequencing (GAWDS) represents a rapid and sensitive method for accessing sequence information. With approximately ten hours of labor, we routinely obtain sequence information on eight segments of DNA from an input of as little as 1 ng of genomic DNA. GAWDS is initiated by polymerase chain reaction (PCR) where at least one of the primers contains a phage promoter such as T7 5' to the complementary sequence. The amplified fragment is then transcribed in vitro to provide an abundance of single-stranded template for reverse transcriptase mediated dideoxy sequencing. We are able to amplify several fragments in one PCR reaction, sequencing multiple regions from one amplification. GAWDS will allow the routine determination of mutations in human genes. We are utilizing this method to define mutations in the factor IX gene of patients with hemophilia B. Eleven segments of the factor IX gene are being sequenced, including the putative promoter site, the coding regions, the splice junction sites, and the termination region with the polyadenylation site. By sequencing these portions of the factor IX gene, it should be possible to determine the mutation in most individuals studied.

H 315 DIFFERENTIAL EXPRESSION OF TWO INTERFERON-BETA INDUCIBLE mRNAS IN INTERFERON SENSITIVE AND RESISTANT HUMAN MELANOMA CELL LINES, Andrew C. Larner, Katsushige Gomi, Lance A. Liotta, and Ruth J. Muschel, Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892.
The expression of two interferon-beta inducible mRNAs (IFN-IND-1 and 2) and the inhibition of c-myc RNA by interferon was examined in interferon-sensitive and resistant human melanoma cell lines. The variant melanoma cell line resistant to the growth inhibitory effects of interferon-beta has a significantly reduced accumulation of mRNAs corresponding to IFN-IND-1 and 2 while the inhibition of c-myc expression by interferon-beta is the same in both the resistant and sensitive cell lines. This comparison has allowed us to separate at least two mechanisms through which interferon-beta regulates the expression of cellular mRNAs. The inhibiton of c-myc accumulation by interferon-beta appears to involve a protein kinase C dependent pathway since interferon inhibits phorbol ester induction of c-myc mRNA. The mechanism by which interferon induces IFN-IND-1 and 2 is not understood. The use of an interferon-gamma resistant cell line has also provided evidence that there are two distinguishable 56,000 dalton polypeptides induced by interferons in these cells; one of these proteins is selectively induced by type I interferons and the other is induced by type II interferons.

H 316 MOLECULAR GENETIC STUDIES IN METHYLMALONIC ACIDEMIA, Fred D. Ledley, Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston TX 77030.
Methylmalonic acidemia (MMA) is an inborn error of organic acid metabolism caused by deficiency of the enzyme methylmalonyl CoA mutase (MCM). This enzyme catalyzes an essential step in the degredation of various branch chain amino acids and odd chain fatty acids. Approximately 1:29,000 newborns have evidence of MMA on newborn urine screening due either to deficiency of the MCM apoenzyme or enzymes required for generation of the adenosylcobalamin cofactor. MCM apoenzyme deficiency can cause widespread metabolic disruptions, mental retardation, and death. A cDNA for human MCM was cloned from a human liver λgt11 cDNA library by antibody screening. A corresponding full length cDNA was subcloned into a eukaryotic expression vector and introduced into COS cells. Cells transformed with the MCM cDNA in a sense orientation exhibited a 2.5-5 fold increase in assayable MCM enzymatic activity. Clones containing the cDNA in an antisence orientation did not increase enzyme activity. The human MCM clone has been used to study MCM expression in fibroblasts from patients with MMA. Seven cell lines from patients with mild MMA (mut^-) or defects in cobalamin metabolism (cbl) had normal amounts of mRNA. In two cell lines from patients with severe MMA (mut^o) hybridizable mRNA was dramatically decreased or absent. Because of the protean clinical manifestations of severe MMA, this disorder may be an ideal candidate for experiments in somatic gene therapy. The present results document the cloning of the MCM gene and demonstrate the feasibility of constituting MCM holoenzyme activity by gene transfer.

H 317 LIVE RECOMBINANT ADENOVIRUS CAN PROTECT CHIMPANZEES AGAINST HEPATITIS B, Massimo Levrero, Annick Ballay, Huub Schellekens^ Pierre Tiollais* and Michel Perricaudet, ER272,IRSC,Villejuif,Institut Pasteur*,Paris,France and TNO^Rijswijk,The Netherlands.

The use of human adenovirus (Ad) as a vector presents several advantages: 1. Ad can be easily propagated in vitro;2. A high copy member of viral genome is present within the cell during replication;3. The genome possesses strong promoters;4. DNA fragment up to 7 kbp may be inserted;5. A live Ad type 4 and 7 has been found to be safe and effective for human use. We have constructed a recombinant virus,derived from Ad5,which arbors the genes of hepatitis B virus (HBV) coding for the surface antigen HBsAg and for the receptor for polymerized albumin (pHSA) under the control of the adenovirus major late promoter (MLP). The recombinant virus directs in infected cells the synthesis and secretion of high levels of HBsAg particles carrying the pHSA receptor. The inoculation of rabbits with the recombinant elicits the synthesis of both anti-HBs and anti-pHSA receptor antibodies. To test the ability of the recombinant virus to protect against hepatitis,it was administered to two chimpanzees. Following a challenge with HBV,over two vaccinated animals,one of them was fully protected. Our study shows for the first time that chimpanzees receiving intravenously an HBV-adenovirus recombinant can be protected against hepatitis B. Although additional scientific information are needed to ensure both efficacy and safety,our results constitute the basis for the development of Ads as a live vaccine for humans.

H 318 SPECIFIC INHIBITION OF β2-MICROBLOBULIN GENE EXPRESSION BY ANTI-SENSE RNA IN MOUSE L-CELLS, Takeshi Matsunaga, Vuokko Tormanen, Unit of Applied Cell and Molecular Biology, University of Umeå, 901 87 Sweden.

It would be very useful to suppress specifically the expression of the β2 micro-globulin (β2m) gene in mice in order to inhibit all the class 1 MHC antigens expressable in early mouse embryos. Along this line, we have constructed synthetic DNA fragments which correspond to two regions the mouse β2m gene and inserted into plasmids where anti-sense RNA can be synthersized by transcription driven by the mouse metallothionein gene promotor in these plasmids. We have obtained a number of mouse L cell clones from the co-transfection with TK gene. Some of the cell clones analysed showed reduced amount of β2m transformed cells. Upon further confirmation of these results, we plan to microinject this DNA construct into mouse eggs to study potential inhibitory effects on mouse embryos.

H 319 REGULATTION OF THE PROMOTER ACTIVITY OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I, Masataka Nakamura, Kiyoshi Ohtani, Shigeru Saito and Yorio Hinuma, Institute for Virus Research, Kyoto University, Kyoto, Japan.

Human T-cell leumemia virus type I (HTLV-I) is a member of the family of retroviruses and appears to be the etiologic agent of adult T-cell leukemia although the virus contains no typical oncogene. The long terminal repeats (LTRs) of retroviruses contain sequence for viral expression and comprise three distinctive domains, U3, R and U5. A novel gene product of HTLV-I, p40, transactivates gene expression from a promoter unit in the U3 region of the HTLV-I LTR. We studied the mechanisms of transactivation by p40 using a transient expression of CAT gene driven by the HTLV-I LTR and a p40 expression vector in Jurkat and K562 cells. Our results showed that an enhancer in the U3 region is activated not only by p40 but also by a tumor promoter TPA that is an activator of protein kinase C, or forskolin that is an activator of adenyl cyclase. We also confirmed that a 21-bp motif three repeats of which are present in the enhancer is mapped for p40 mediated transactivation. However, two out of three enhancer subfragments containing the 21-bp motif are not responsive to p40 in K562 cells. The enhancer element can bind nuclear factor(s) from both p40-expressing and -unexpressing cells. In addition, we showed that other regulatory element is present in the R region. Function of this element does not require p40 and is distinct from a classical enhancer in that the activity of the second element is strictly dependent on its location. These results suggest that multiple regulatory mechanisms are involved in promoter activity of HTLV-I.

H 320 EXPRESSION OF GENES INSERTED AT THE HUMAN -GLOBIN LOCUS BY HOMOLOGOUS RECOMBINATION, *Asit K. Nandi, *Raymond S. Roginski, +Ronald G. Gregg, +Oliver Smithies, *Arthur I. Skoultchi, +Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706 and *Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461.
Introduction of human α-and β-globin genes into mouse erythroleukemia (MEL) cells leads to regulated expression during chemically induced in vitro differentiation of the cells. Chromosome mediated transfer of human globin genes by cell fusion with MEL cells results in correctly regulated expression to a level comparable to endogenous mouse globin genes. Inducible expression has also been observed with cloned human β-globin genes during MEL differentiation but the degree of induction is variable and expression per gene copy is low. Smithies et al. (Smithies, O. et al. Nature 317, 230-234, 1985) have inserted a modified β-globin gene and a neo gene with SV40 transcription signals into the chromosomal -globin locus in human chromosome 11 in MEL-human cell hybrids. We have measured the expression of both inserted genes when they are located in the modified β-globin locus and also at random integration sites. We find from such studies that chromosomal position is important for achieving proper regulated expression during erythroid differentiation.

H 321 MOLECULAR ANALYSIS OF CELL-TYPE SPECIFIC EXPRESSION OF THE HUMAN LYSOZYME GENE Christoph W.B. Peters, Ulrich Kruse, Martin Vingron, Renate Pollwein and Albrecht E. Sippel, ZMBH, University of Heidelberg, D-6900 Heidelberg, W.-Germany
The mechanisms of cell-type specific expression of the lysozyme gene have primarily been studied in chicken (Sippel et al., in Structure and Function of Eucaryotic Chromosomes, Edited by W. Hennig, Springer,Heidelberg/Berlin). To investigate the control elements of cell-type specific expression of the human lysozyme gene we have isolated the human lysozyme gene by hybridization with a partial cDNA clone (provided by P. Swetly and W. Spewak). Sequence analyses revealed the complete coding region of the human lysozyme on four exons seperated by three introns together spanning approximately 5 kb. The deduced amino acid sequence is identical with the published protein sequence (Jolles et Jolles, Hel.Chi.Act., Vol 54, 2668 - 2675, 1971) and preceded by a putative signal peptide of 18 amino acids. Two putative polyadenylation signals have been localized 42 bp and 57 bp downstream of the translation stop codon, respectively. The start of transcription has been mapped 28 bp upstream of the ATG initiation codon by S_1 nuclease mapping using total RNA from human myeloid cell lines U937 and HL60. Sequence analysis of the promotor region revealed a high degree of similarity with the chicken lysozyme promotor in the vicinity of the TATA box only. Clones of the human lysozyme promotor and 5' upstream region fused to the firefly luciferase gene are tested in transient expression assays for the identification of cell-type specific regulatory elements. As an alternative approach to identify such elements we map DNAaseI hypersensitive sites in the chromatin of the human lysozyme gene in cells expressing (U937) and in cells not expressing (HepG2) the gene.

H 322 **THE REGULATION OF ENHANCER ACTIVITY IN MOUSE BONE MARROW CELLS IN *VIVO* AND IN EMBRYONIC CARCINOMA CELLS,** Paul Robbins and Richard Mulligan, Whitehead Institute, Cambridge, MA 02142.
Retroviral vectors have been used to introduce selectable marker genes into mouse bone marrow; however, expression from the LTR of Moloney-based vectors has been extremely low in long term reconstituted animals. We have attempted to overcome the block to viral gene expression by altering the viral regulatory sequences. We have replaced the Moloney enhancer element with several heterologous enhancer sequences shown to function in embryonic carcinoma cells (SV40,, Polyoma F9.1, MPSV) or with a hematopoeitic cell-specific enhancer sequence (mouse immunoglobulin heavy chain). The modified viral LTRs were tested for expression in embryonic carcinoma cells as well as in transplanted mouse bone marrow cells. The replacement of the viral enhancer did not significantly increase expression in long term reconstituted animal or in F9 cells. We have therefore been dissecting the *cis*-acting sequences and identifying the *trans*-acting factors required for the negative regulation of enhancer activity in F9 cells. We have shown that the octamer sequence contained within the heavy chain and SV40 enhancers negatively regulates enhancer activity and that the negative regulation may be mediated by a distinct octamer-binding protein (B4). Furthermore, we have shown that the Polyoma F9.1 point mutant allows binding of a factor (Act) present in F9 cells as well as in a variety of cell types. The same point mutation also allows binding of NF-1 in fibroblast cells to an overlapping sequence. We do not, however, detect the presence of NF-1 in F9 cells. Intriguingly, Adenovirus E1A 12S and 13S cDNAs appear to repress the level of NF-1 in 3T3 cells while the 13S cDNA activates the level of Act. We are now examining the effect of the octamer mutation on SV40 enhancer activity as well as the effect of NF-1 and Act binding sequences on expression from the Moloney LTR in the bone marrow of long-term reconstituted animals. In addition, we are using an internal tk promoter vector to analyze the effects of the various *cis*-acting sequences on expression.

H 323 CLONING OF A NEW BRAIN Ca^{++}/CALMODULIN-DEPENDENT PROTEIN KINASE cDNA USING A LIGAND PROBE, James M. Sikela and William E. Hahn, University of Colorado School of Medicine, Denver, CO 80262.

We have previously reported the isolation of a cDNA encoding a Ca^{++}-dependent calmodulin binding protein that appears to be present only in brain (PNAS 84:3038-3042, 1987). The cDNA was isolated using ^{125}I-calmodulin to screen a λgt11 mouse brain expression library. When the cDNA sequence was used to search DNA and protein databases no significant matches were found suggesting that it did not correspond to any known gene or protein. Because the original cDNA was partial-length, several strategies designed to produce additional cDNA sequences were utilized, but were not successful, suggesting that such sequences may not clone efficiently or at all. We have now used a genomic restriction fragment thought to contain additional upstream sequences to screen a cDNA library that was constructed using, as a primer, a synthetic oligonucleotide complementary to part of the original cDNA. A single 200 bp cDNA was isolated that occurred at a frequency 200-fold less than the original cDNA, which was isolated from an oligo dT-primed library. Sequencing of this cDNA revealed a deduced opening reading frame (ORF) that displays significant sequence similarity with the catalytic domain of several known serine kinases. Comparison of the ORF sequence with several Ca^{++}/calmodulin-dependent protein kinases (CAM kinases) indicates that the position of the calmodulin binding site relative to the catalytic domain is the same as that found in the other CAM kinases. Also the calmodulin binding site of our clone and those of the α and β subunits of the Type II CaM kinase have 8 consecutive amino acids that are identical (and 9 of 10).

H 324 A BOVINE PAPILLOMA VIRUS VECTOR UNDERGOES HIGH FREQUENCY HOMOLOGOUS RECOMBINATION WITH THE HOST GENOME IN MOUSE FIBROBLASTS. David Strehlow and Paul Berg, Stanford University, Stanford,Ca. 94306

Bovine papilloma virus vectors replicate as autosomal elements in rodent fibroblasts.We constructed a vector with the complete BPV genome, a hygromycin transcription unit, sequences from pBR322, and a defective neomycin gene to monitor homologous recombination. By introducing this vector into 3T6 mouse fibroblasts which contained an integrated, complementary defective neo gene, we found a 100 fold increase in the frequency of homologous events when compared to non-replicating vectors in the same cells. Reconstruction of the normal neo gene was verified by southern analysis of whole cell DNA and recovery of neo DNA by kanamycin selection in E. coli. Greater than 90% of the events analyzed where gene conversion events , as determined by Southern analysis of the reciprocal partners of the recombination event. 95% of all events examined were wild type for neo in the autosomal DNA.

H 325 MURINE HISTIDINEMIA AS A MODEL FOR GENE THERAPY: CLONING OF A cDNA FOR HISTIDASE. R.G. Taylor, E. Sexsmith, M. Lambert, D.J. Mahuran, H. Kacser, G.Bulfield and R.R. McInnes. Hospital for Sick Children, Dept. of Genetics, Toronto, Canada, and University of Edinburgh, Scotland.

The catabolic enzyme histidase is deficient in murine and human histidinemia. Histidinemic mice (*his/his*) are representative of clinically important enzymopathies (e.g. PKU) in which there is an excess of a freely diffusible substrate. To investigate the ability of somatic gene therapy to reverse the biochemical phenotype of disorders of this type, histidinemic mice are an excellent model: the enzyme is a cytoplasmic homopolymer, and the cDNA can be intoduced into any readily transplantable cell type (e.g. bone marrow, fibroblasts). As the initial step in this project we isolated histidase cDNA clones from a rat liver λgt11 library using an affinity-purified polyclonal rabbit antiserum. The specificity of the purified antiserum was confirmed by its ability to recognize the 75 kd histidase monomer on an immunoblot of C57BL mouse liver, whereas only a trace band was seen in histidinemic mouse liver. We used the initial clone to obtain a 2.1 kb cDNA that hybridized to a major mRNA of 2.4 kb in mouse liver, but detected no mRNA in fibroblasts, brain, heart, kidney, or placenta. This distribution of expression agrees with enzymatic data in the literature. In addition, a 9 amino acid peptide, isolated by reverse phase HPLC chromatography of a tryptic digest of the reduced and alkylated protein, has a corresponding nucleotide sequence in a predicted open reading frame of the cDNA, firmly establishing its identity. A full length histidase cDNA will be isolated and introduced *in vitro* into cells cultured from histidinemic mice (e.g. fibroblasts, hepatocytes) using retroviral vectors. This model will allow quantitative analysis of the effectiveness of somatic gene therapy for this type of disease simply by measurement of the plasma histidine, permitting *in vivo* studies that must be done before gene therapy can be systematically used in patients.

H 326 CHARACTERIZATION OF THE CIS AND TRANS-ACTING ELEMENTS ESSENTIAL FOR RAT INSULIN II GENE EXPRESSION; Ming-Jer Tsai, Young-Ping Hwung, David Crowe, Lee-Ho Wang, and Sophia Y. Tsai, Dept. of Cell Biology, Baylor College of Medicine Houston, TX 77030.

To define the cis-acting elements in the rat insulin II gene we constructed a series of linker scanning mutants throughout the enhancer and promoter regions. These linker scanning mutants were transfected into insulin producing cells and three mutants (LS-261/252, LS-102/91 and LS-54/45) displayed drastically reduced levels of CAT activity. Therefore, at least three regions are essential for the insulin promoter function.

To identify the trans-acting factor which interacts with the promoter element we carried out gel retardation and DNase I footprinting analyses. A binding activity in the nuclear extract of HIT cells and HeLa cells protects a region between -45 and -60 from DNase I digestion. Purification of this binding activity from HeLa cell extracts shows that it is co-purified with the COUP (Chicken Ovalbumin Upstream Promoter) transcription factor, a DNA-binding protein which is required for transcription of the chicken ovalbumin gene. Although, the binding sequence of the insulin and the ovalbumin promoters are quite different, several lines of evidence suggest that they are the same protein. First, the ovalbumin and insulin promoters compete with each other for the binding of the COUP transcription factor as assayed by gel retardation assay. Second, the COUP transcription factor renatured from individual SDS gel bands, binds to both the ovalbumin and the insulin promoters. Third, methylation interference experiments show that the binding sites shares some purine contact points. However the phosphate contacts and DNase I footprinting covering regions of the COUP binder on these two promoters are quite different.

H 327 MAPPING AROUND THE CYSTIC FIBROSIS LOCUS. *Wilfried Bautsch (1), Dietmar Grothues (1), Joachim Hundrieser (2), Norbert Ponelies (3), Andreas Claaß (4), Karl-Otto Greulich (3), Karl-Heinz Grzeschik (5), Gerhard Rechkemmer (6), Eberhard Frömter (7), Günter Maaß (1) and Burkhard Tümmler (1). Zentrum Biochemie (1) and Humangenetik (2), Medizinische Hochschule Hannover, Physikalisch-Chemisches Institut, Universität Heidelberg (3), Universitätskinderklinik Kiel (4), Humangenetisches Institut, Universität Marburg (5), Physiologisches Institut, Tierärztliche Hochschule Hannover (6), Physiologisches Institut, Universität Frankfurt (7), FRG.*

For the construction of a physical genome map around the cystic fibrosis (CF) locus single gene markers were isolated from metaphase 1:7q2-qter chromosomes by Laser - microdissection and subsequent microcloning. Probes were localized by hybridization to somatic cell deletion hybrids and long-range restriction mapping. Pedegree studies in 90 German CF families revealed several recombinants between the closely linked marker loci D7S8 and MET. Genomic clones of the chromosomal region were analyzed for expression by screening Northern blots and N and CF libraries from exocrine tissues. - The basic defect in CF is associated with non-responsiveness of the apical chloride channels of epithelial cells to β_2-adrenergic agonists. mRNA from N and CF nasal polyps and nasal epithelial cells was injected into Xenopus laevis oocytes and assayed for stimulus-dependent chloride channel activity by electrophysiological means. mRNA preparations from dog trachea and colon epithelial cell lines served as controls. The actual results of the assays on gene expression and complementation will be presented at the meeting. *Supported by the Deutsche Forschungsgemeinschaft.*

H 328 INSERTION OF AN SV40 ENHANCER EXPANDS SPECIES AND CELL TYPE HOST RANGE OF THE HUMAN POLYOMAVIRUS, JCV, Dominick Vacante, Sidney Houff, Gregory Elder and Eugene Major, NINCDS, NIH, Bethesda, MD 20892

In order to augment the production of the human polyomavirus, JCV, for potential animal studies, a chimeric polyomavirus genome was constructed. The regulatory sequences of SV40 were inserted into the agno region of the JCV genome on the late side of the JCV 98-bp tandem repeats. This construction would not alter the primary sequences of the JCV T and capsid proteins. Upon DNA transfection into human fetal brain cells in culture, the tissue specific target for JCV infection, virions were produced that demonstrated genetic rearrangements in the constructed region. Sequence analysis of a viable clone showed a 298-bp deletion but retained the functional sequences for the JCV replication origin, 78-bp of one 98-bp repeat, 32-bp of one SV40 72-bp repeat and one intact 72-bp repeat. This genome, however, had an extended host range, not restricted by species or cell type, producing infectious virus in both human fetal brain and embryonic kidney as well as in rhesus fetal and adult glial cells. Analysis of the RNA transcripts suggested that the early promoter consisted of JCV sequences while the late promoter consisted of SV40 sequences. The JCV T protein produced by this virus showed characteristics of the wild type JCV T protein since it co-migrated with the wild type T protein in denaturing gels and did not complex with the cellular p53 protein. These data suggest that only the noncoding sequences of the JCV regulatory region control the host range of the virus. Because of its extended species host range, this virus has been used to develop a sub-human primate model for progressive multifocal leukoencephalopathy, the acute demyelinating disease in the human brain caused by JCV.

H 329 AN ANDROGEN RECEPTOR RELATED PROTEIN IS MISSING IN CELLS FROM PATIENTS WITH ANDROGEN RESISTANCE SYNDROME. Klaus Wrogemann, Denise Belsham, Fred Pereira, Eduardo Rosenmann, Edward Nylen, Morris Kaufman and Leonard Pinsky. Departments of Biochemistry and Human Genetics, University of Manitoba, Winnipeg, Manitoba, and Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada.

We have discovered an abundant 56K (more accurately 58K) protein in genital skin fibroblasts (GSF), (Nature 298, 563 (1982); 304, 740 (1983). The protein is watersoluble and present in the soluble fraction but not in nuclei. It was detected in all of 23 GSF cell strains of both sexes, but not in 30/32 non-GSF strains. In contrast, GSF cells from 13/15 patients with complete androgen resistance do not have the 56K protein. The other 2 patients, also with no detectable androgen receptor binding activity, do express the protein. Thus, the protein cannot be androgen induced. Photolytic labeling of intact cells with ^{3}H-methyltrienolone (MT), followed by 2-D electrophoresis, shows that the 56K protein is specifically and preferentially labelled with MT, but with a lower affinity than is known for the AR. The hierarchy of competing steroids is the same as for the AR. A polyclonal antiserum against denatured 56K protein detects the 56K protein on Western blots of 2-D gels of cytosol, and CRM of higher molecular weight in isolated nuclei. The antiserum so far could not be used to precipitate native AR complexes or native 56K protein itself. In vitro translation experiments indicate that the protein is synthesized directly and not as a larger precursor. We postulate that this protein, although not the AR itself, is synthesized from the AR gene. (Supported by MHRC, MRC and MDAC).

H 330 DIFFERENTIAL REGULATION OF ALA SYNTHASE GENES IN DBA/2 MOUSE
Ellora Young and Peter Dierks, Dept. of Biology, Gilmer Hall, Univ.of VA, Charlottesville, VA 22901

Heme is required for many essential functions in mammalian organisms. It functions as the prosthetic group of cytochromes in electron transport, is involved in a wide variety of cytochrome P450 mediated biotransformation reactions, and functions as the oxygen carrier for hemoglobin in erythrocytes. The rate of heme biosynthesis in the cell appears to be controlled by the activity of the first enzyme in the pathway--aminolevulinate synthase (ALAS). In addition to a general feedback repression mechanism by heme, ALA synthase appears to be developmentally regulated in erythrocytes prior to globin induction. These observations, coupled with claims that different ALAS isozymes are expressed in hepatic and erythroid cells, have led to the suggestion that different genes are used to encode ALAS in these two tissues.

We have isolated two distinct ALA synthase cDNAs and their genes in DBA/2 mouse and can demonstrate that the expression of these genes *in vivo* is differentially regulated in erythroid and hepatic tissues. Expression of the α gene is induced during erythroid differentiation while the β gene is specifically derepressed in the liver in response to drugs that induce hepatic porphyria. We are currently utilizing a murine erythroleukemia cell line and a murine hepatoma line as model systems to decipher the many regulatory interactions between the physiological state of the cell and these two genes.

H 331 MOLECULAR IDENTIFICATION OF A HUMAN S-PHASE GENE FOLLOWING DNA-MEDIATED GENE TRANSFER, Eldad Zacksenhaus and Rose Sheinin, University of Toronto, Toronto, Ont. M5S 1A8.

We have previously described a temperature sensitive (ts) mouse L-cell, ts A1S9, defective in a gene which is involved in nuclear but not polyoma viral DNA replication. We employ DNA-mediated gene transfer methodology to identify and subsequently isolate the A1S9 gene. Human DNA sequences were introduced into ts A1S9 cells together with a selectable neomycin gene carried on the plasmid pSV2neo. Primary, secondary and tertiary transformants expressing both the transferred human A1S9 gene and the neomycin gene were isolated in the presence of the drug geneticin-sulfate at 39^{0}C, the non-permissive temperature. These were analysed by Southern blot hybridization with a human alu repetitive sequence, Blur-8, which we subcloned from the original pBR322 vector into an M13 derivative in order to avoid cross-hybridization with endogenous pSV2neo sequences present in the contransformant genomes. All the transformants have retained common sets of alu-containing restriction fragments which span a region of at least 28 kb and are likely to be accommodated within or in proximity to the transferred gene. This should allow the retrieval of the human A1S9 gene from a genomic library, currently being constructed from one of the secondary transformant DNA, by screening with the human-specific alu repetitive sequence probe.