TRANSGENES, DEVELOPMENT AND DISEASE

Organizers: Heiner Westphal and William Drohan

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Recombination and Gene Regulation

B 001 THE RECOGNITION OF SEQUENCE HOMOLOGY BY RECOMBINATION PROTEINS, Peggy Hsieh, Carol S. Camerini-Otero, Ryoiti Kiyama and R. Daniel Camerini-Otero, Genetics and Biochemistry Branch, NIDDK, National Institutes of Health, Bldg. 10, Rm. 9D-15, Bethesda, MD 20892.

In order to dissect the biochemical steps involved in homologous recombination we have chosen to focus on a key early step: strand exchange between homologous parental DNAs. Until a few years ago only two proteins responsible for this had been studied in detail: RecA from *E. coli* and uvsx from bacteriophage T4. Over the last few years we have reported on the purification and characterization of similar proteins from nuclear extracts of human cells (1) and embryos of *D. melanogaster* (2). We will report on our progress and that of other groups on the cloning of the genes for these recombinases.

Recently, we have shown that the structure of the protein-free intermediate in strand-exchange is most likely that of a three-stranded nucleic acid (3). In essence, we propose that the recombinases promote conformational changes in the structure of the duplex and single strand DNAs that allow them to hybridize to each other. In this way the DNAs themselves mediate the recognition of homology. We will report on a model we have built of the precise hydrogen-bond interactions between the third strand and the duplex. We will also report on our progress in critically testing this model: e.g., chemical probing of reactive groups in the three strands. In addition, in order to confirm the plausibility of certain base-base interactions in the model of the enzymatically formed three-stranded structures, we have used simple sequences to extend the range of sequences that can non-enzymatically be induced to form triplexes. Although, for the most part, such triplexes have only been described between two polypyrimidine (Py) strands and one polypurine (Pu) strand, we have been able to form triplexes under physiological conditions with sequences that contain all four bases and with one Py and two Pu strands.

Finally, in order to study the initial (synaptic) interaction between homologous DNAs and recombinases we have established an assay to detect homology-dependent ternary interactions between single strand DNA, double strand DNA and the *E. coli* RecA recombinase protein. Our data shows that RecA, and most likely eukaryotic recombinases, appear to nucleate pairing *in vitro* by sampling very short, discrete regions of DNA. In fact, it appears that the initial pairing event mediated by RecA involves less than one turn of the DNA helix.

(1) Hsieh, P., Meyn, M.S., and Camerini-Otero, R.D. (1986). *Cell 44,* 885-894.
(2) Eisen, A., and Camerini-Otero, R.D. (1988). *Proc. Natl. Acad. Sci. USA 85,* 7481-7485.
(3) Hsieh, P., Camerini-Otero, C.S., and Camerini-Otero, R.D. (1990). *Genes Dev.*, in press.

B 002 Mechanisms of transcriptional activation by POU homeodomain proteins Oct-1 and Oct-2. Winship Herr, Rajeev Aurora, Michele Cleary, Gokul Das, Seth Stern, and Masafumi Tanaka. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Many genes that control development encode sequence-specific DNA binding proteins that regulate transcription, such as those of the homeodomain class. Frequently, homeodomain proteins display quite similar DNA binding specificities but effect very different developmental programs. To understand the mechanisms by which homeodomain proteins can bind to the same DNA sequence and yet differentially regulate transcription we study transcriptional activation by the mammalian octamer-motif (ATGCAAAT)-binding proteins Oct-1 and Oct-2. The ubiquitously expressed Oct-1 protein is implicated in regulation of small nuclear RNA genes (snRNA) transcribed by RNA polymerase II (e.g. U2 snRNA) and RNA polymerase III (e.g. U6 snRNA), whereas the lymphoid Oct-2 protein is implicated in cell-specific regulation of typical mRNA promoters such as the immunoglobulin gene promoters. Oct-1 and Oct-2 are closely related proteins that belong to the POU class of homeodomain proteins. POU homeodomain proteins are characterized by a large region (150 to 190 amino acids) of sequence similarity, called the POU domain; this domain contains two subdomains, an N-terminal POU-specific domain and a C-terminal homeodomain. Both of these regions are responsible for the full sequence-specific DNA binding activity of these proteins.

Our studies of the activation potentials of Oct-1 and Oct-2 have revealed that these two proteins carry different types of activation domains, which lie outside of the POU DNA binding domain. The C-terminus of Oct-2 specifies activation of TATA box - containing mRNA promoters whereas the C-terminus of Oct-1 is unique because it fails to activate mRNA promoters but instead can activate the class of promoters represented by the different U2 snRNA promoter. Thus, here, differential transcriptional activation is specified by protein domains outside of the DNA binding domain. This result contrasts with the herpes simplex virus (HSV) activator VP16 (Vmw 65) which differentially activates transcription by Oct-1 and Oct-2 by discriminating between the very similar (90% conserved) Oct-1 and Oct-2 DNA binding domains.

VP16 is a HSV virion protein that activates the HSV immediate early genes by associating with Oct-1 and a second cellular factor to form a multiprotein DNA complex. VP16 serves as an adaptor that, by virtue of a very strong acidic activation domain, converts Oct-1 into an activator of mRNA promoters. VP16 does not, however, associate with Oct-2 and this is because the Oct-1 and Oct-2 homeodomains differ at 7 positions within the solvent exposed non-DNA binding portion of the homeodomain. VP16 association with Oct-1 stabilizes the interaction of Oct-1 with certain binding sites called the TAATGARAT; this stabilization results in a differential response of cis acting elements to Oct-1 activation by itself as opposed to in association with VP16.

B 003 RAG-1 AND RAG-2: ADJACENT GENES THAT SYNERGISTICALLY ACTIVATE V(D)J RECOMBINATION, David G. Schatz, Marjorie A. Oettinger, Carolyn Gorka and David Baltimore, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142. The ordered rearrangement of immunoglobulin and T cell receptor V, D and J gene segments to produce functional immune receptors is a central process of lymphoid development that occurs only in pre-B and pre-T cells. While the order of rearrangements is known and the cis-acting recombination signal sequences have been defined, little is known about the recombinational machinery, its regulation, or the factors governing its expression. Transfection of genomic DNA into NIH 3T3 cells, a fibroblastoid line that lacks V(D)J joining activity, can stably induce the expression of the V(D)J recombinase. Isolation of the genetic locus responsible for this induction led us to the identification of the Recombination Activating Gene (RAG-1), a lymphoid-specific gene whose expression correlates precisely with recombinase activity. RAG-1 inefficiently induces V(D)J recombinase activity when transfected into fibroblasts. In trying to understand the reason for this inefficient induction, we identified a second gene, RAG-2, adjacent to RAG-1. As with RAG-1, transfection of RAG-2 into fibroblasts activates V(D)J recombination poorly; however, in comparison to using either gene alone, cotransfection of RAG-1 and RAG-2 results in at least a 1000-fold increase in the frequency of recombination. The 2.1 kb RAG-2 cDNA encodes a putative protein of 527 amino acids whose sequence is unrelated to that of RAG-1. Like RAG-1, RAG-2 is conserved between species that carry out V(D)J recombination and its expression correlates precisely with that of V(D)J recombinase activity. The RAG locus is unusual not only because the two convergently transcribed genes are located just 8 kb apart, but also because most, if not all, of the RAG-1 and RAG-2 coding and 3' untranslated regions are contained in single exons. RAG-1 and RAG-2 either serve as regulators of a pathway leading to the expression of the V(D)J recombinase or, more likely, directly encode lymphoid-specific components of the recombination machinery. We are attempting to distinguish between these possiblilities through a combination of biochemical and in vivo functional studies. In addition, we are trying to understand 1) the regulation of RAG-1 and 2 expression and 2) how RAG-1 and 2 carry out the complex recombination reaction (assuming that they indeed encode the V(D)J recombinase).

Regulation of Embryonic Development

B 004 COORDINATE EXPRESSION OF THE MURINE HOX-4 COMPLEX HOMEOBOX-CONTAINING GENES DURING PATTERN FORMATION ALONG THE BODY AXES, Vincenzo Zappavigna*+, Juan-Carlos Izpisúa-Belmonte*, Hildegard Falkenstein*, Armand Renucci*, Pascal Dollé*, Cesare Peschle*+ and Denis Duboule*, *European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg and +Istituto Superiore di Sanita,00151, Roma Italy.
The study of patterning and development of the vertebrate limbs is one of the most fascinating ways to investigate morphogenetic mechanisms. The homeobox-containing genes of the HOX-4 complex are expressed in different but overlapping domains in both the trunk and the limbs during murine development. The more 5' the position of these genes in the complex, the later and more distal is their expression. In the limbs, antero-posterior differences are also observed. A model is proposed that accounts for the establishment of these expression domains in relation to the existence of a morphogen, possibly retinoic acid, released by the zone of polarizing activity. In order to further investigate the validity of this proposal, the chicken Hox-4 complex was cloned and extensively characterized. It shows a structural and functional organization identical to its mouse counterpart. In collaboration with Dr.C. Tickle and Prof.L. Wolpert (London), the expression of several of these chicken genes is studied in experimentally manipulated limbs hopefully providing new insight into the function of these homeogenes during morphogenesis. Comparison of these observations with the expression patterns of the genes of Hox complexes in the early embryo suggests that similar molecular mechanisms are involved in the positional signalling along the axes of both the embryonic trunk and the fetal limbs. These coordinate expression patterns strongly suggest that transcriptional regulatory mechanisms exist within the Hox network. We have addressed this question by studying a sequence located in the promoter of the Hox-4.4 gene. This DNA fragment (about a 100 bp large) was isolated on the basis of its conservation in both human and mouse promoters. It contains several DNA motives which are able, in vitro, to bind the various Hox-4 proteins. In transfected cultured cells, this upstream sequence is able to mediate transactivation of a reporter gene by the products of different Hox-4 genes.

B 005 **MURINE DEVELOPMENTAL CONTROL GENES**

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Different strategies have been employed to isolate genes implicated with a regulatory role during mouse development. Gene families isolated on the basis of homology to motifs within Drosophila control genes or human transcription factor genes, namely homeobox (Hox), paired-box (Pax) and POU genes will be described with respect to gene organisation, structure and expression patterns. Functional analysis of these genes can be approached both on a molecular level in vitro and in vivo using available mouse mutants or transgenic mice. Experiments aimed at generating gain or loss of function mutations by random or targeted introduction of transgenes will be described. Models derived from these studies promise to reveal steps of the developmental control on a genetic level.

B 006 MOLECULAR GENETIC AND MUTATIONAL ANALYSIS OF GENES INVOLVED IN PATTERN FORMATION, **A.L. Joyner**, K. Herrup*, W. Skarnes, W. Wurst, A. Auerbach and J. Rossant, Samuel Lunenfeld Research Institute of Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5 and *E.K. Shriver Institute, 200 Trapelo Road, Waltham, MASS, 02254, U.S.A.

As an approach to gain insight into the genetic control of pattern formation in mammals, we have been studying the two *engrailed*-like genes *En-1* and *En-2*. A comparative analysis of *En* genes from different vertebrates has demonstrated that the proteins encoded by these genes as well as their expression patterns during early embryogenesis have been highly conserved during evolution. This suggests a conserved role for these genes in development. *En-2* expression is restricted to a band of cells spanning the mid/hindbrain junction during early embryonic development and progresses to a cell type specific pattern in the adult pons, cerebellum and substantia nigra. *En-1* appears to be co-expressed with *En-2* early in embryogenesis and in the adult brain. The one major exception is in the developing cerebellum, where *En-2* alone is widely expressed until after birth when it becomes restricted to the granule cell layer. *En-1* is also expressed during midgestation in spatially restricted patterns in the spinal cord, somites and limbs, suggesting additional role(s) for *En-1* in patterning outside the brain. To begin to determine whether the mouse *En* genes, like their *Drosophila* counterparts, play a controlling role in CNS pattern formation we have made mice containing a deletion of the *En-2* homeobox via homologous recombination in mouse ES cells. Mice homozygous for this mutation are viable, show no obvious behavioral defects, but show a distinct set of alterations in the patterning of the folia of the cerebellum. This observed cerebellar specific defect, in the absence of an embryonic phenotype, may be explained by redundancy between the two structurally related *En* genes that are co-expressed during early embryogenesis. We are currently attempting to test this by creating a targeted mutation in *En-1* and analyzing the phenotypes of mice lacking *En-1*, or *En-1* and *En-2*. In addition, vectors that express the *En-2* protein ubiquitously are being introduced into embryos to test whether an *En* protein can alter the fate of cells that normally do not express *En* genes. Finally, in order to identify new genes that are involved in embryogenesis, we have been generating mouse mutants via random insertion of a "gene trap" reporter construct in ES cells. The first mouse that we have analysed contains a *lacZ* insertion in a gene expressed in spatially restricted patterns during early embryogenesis. Mice homozygous for this mutation die at birth, thus defining a new developmental mutant. Funded by the MRC and NCI of Canada and the NIH.

B 007 THE HOX-2 HOMEOBOX FAMILY: GENE REGULATION AND PATTERNING OF THE VERTEBRATE HEAD. Robb Krumlauf, Paul Hunt, Jenny Whiting, Stefan Nonchev Heather Marshall, Mai Har Sham, Ian Muchamore, Nancy Papalopulu and David Wilkinson Lab of Eukaryotic Molecular Genetics, NIMR, The Ridgeway, Mill Hill, London NW7 1AA

The Hox-2 homeobox cluster represents a family of nine genes which have patterns of expression suggesting they have a role in axial pattern formation in the vertebrate embryo. There is a correlation between the order of the genes along the chromosome and their boundaries of expression in mesoderm and ectoderm. We previously found that some of the boundaries of expression in the central nervous system mapped within the hindbrain to important segmental units of organisation termed rhombomeres. Hox-2 genes had limits of expression which varied in a two rhombomere periodicity consistent with a role in specification of the identity these segmental units. We have now found that members of other Hox clusters also have limits of expression which correlate with specific rhombomeres and can begin to define the array of Hox genes that may be necessary to provide positional information to the developing hindbrain. We have also extended this analysis to the cranial neural crest. In the head derivatives of the neural crest contribute to the connective tissue and bone of the face, unlike crest from the trunk. Grafting experiments in chicken embryos by Noden have shown that cranial neural crest has a potential to specify structures in a position dependent manner, because it will form bone and cartilage characteristic of it site of origin when grafted into new sites. Hox-2 genes are expressed in cranial neural crest associated with specific visceral arches. From our results it appears that the Hox-2 genes are part of the molecular signalling process that provides the imprinting information for both the rhombomeres and cranial neural crest. We have begun to examine the basis of the spatial and temporal regulation of the Hox-2 cluster using transgenic mice. A lacZ reporter gene has been fused to several Hox-2 members and used to generate transgenic mice. We have successfully reconstructed the normal patterns of expression for several of the genes and begun to perform deletion analysis to map the elements responsible for spatial expression. Multiple elements drive expression in the neural tube and one isolated element from the Hox-2.6 gene is responsibe for rhombomere restricted expression in the hindbrain. We are currently using these regulatory elements to alter the expression patterns of other members of the Hox-2 cluster, in an attempt to generate dominant affects in transgenic mice and directly test for functional roles in positional specification.

Thymus Development

B 008 CD4 AND CD8 IN T CELL DEVELOPMENT, Dan R. Littman, Department of Microbiology & Immunology, University of California, School of Medicine, San Francisco, CA, 94143, and Howard Hughes Medical Institute, San Francisco, CA, 94143. The CD4 and CD8 glycoproteins are expressed on functionally distinct subsets of T lymphocytes. During development in the thymus, these molecules are thought to have important signalling roles that contribute to the selection of appropriate T cell receptor repertoires. CD4 and CD8 both interact directly with MHC molecules via their external domains and bind the cytoplasmic tyrosine kinase, $p56^{lck}$, through their cytoplasmic domains. We have evidence that, for effective activation of T cells, CD4 and CD8 must interact with the same MHC molecule as that which is recognized by the T cell receptor. In addition, we have demonstrated that the interaction of CD4 with the tyrosine kinase is critical for signal transduction in antigen specific T cell hybridomas. We are now using transgenic mouse models to study the significance of these interactions in positive selection and clonal deletion of thymocytes. These analyses are also aimed at identifying the mechanism through which double positive cells give rise to mature single positive cells.

B 009 TRANSGENIC MOUSE MODEL OF POSITIVE AND NEGATIVE SELECTION OF T CELLS, Dennis Y. Loh, Howard Hughes Medical Institute and Departments of Medicine, Genetics, and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110
The antigen specific T cells display three characteristics. Firstly, they display antigen specificity determined primarilty by the primary sequence of the T cell receptor. Secondly, they show immunological self-tolerance or lack of reactivity against antigens present in the organism in which the T cells developed. Thirdly, they recognize antigens only in the context of self major histocompatibility complex products, a phenomenon called MHC restriction. To understand these important phenomena of the immune system, we have developed a T cell receptor transgenic mouse model. Through the use of these mice, we have elucidated how the interaction between the T cell receptor and the MHC molecules determine cell fate of the developing thymocytes. The three primarily fates that have been observed include: (1) apoptosis or programmed cell death when the receptor is self-reactive in the organism; (2) maturational arrest when the receptor cannot interact with the MHC at all; and (3) maturation and exportation of normal single positive T cells into the periphery that has acquired MHC restriction when the strength of interaction is appropriate. The molecular basis of these diverse cell fate will be presented.

B 010 DEVELOPMENT OF γδ T CELLS, Susumu Tonegawa, Howard Hughes Medical Institute at Massachusetts Institute of Technology, Center for Cancer Research and Department of Biology, Cambridge, MA 02139.
Studies using both γδ TCR transgenic and transfection studies demonstrated that a DNA element (silencer) which is associated with the γ gene represses its transcription in αβ lineage cells. Further analysis of γδ TCR and αβ TCR transgenic mice strongly suggested the developmentally controlled activation of the silencer machinery plays a critical role in the separation of the αβ and γδ T cell lineages.
γδ TCR transgenic mice has also been extremely useful in analyzing the development of self-reactive γδ T cells. In contrast to self reactive αβ T cells which are deleted in the thymus, at least some anti-self γδ T cells accumulate in the thymus in a normal number and emigrate to spleen. However, these T cells are in the state of anergy: they cannot proliferate in response to self antigens and cannot produce IL-2, suggesting that the two types of T cells are under the control of distinct developmental programs.

B 011 THYMIC AND POST-THYMIC SELECTION OF THE T CELL REPERTOIRE, Harald von Boehmer, Basel Institute for Immunology, CH-4005 Basel, Switzerland. The antigen-receptor on αβ T cells controls T cell development at various stages. On pre-T cells the β TCR chain is incorporated into the membrane without any other known TCR chain and components of the CD3 complex. As a consequence, the cell starts to express CD4 and CD8 genes and the α TCR locus becomes transcriptionally active

On immature $CD4^+8^+$ thymocytes the αβ TCR^- CD3 complex needs to bind to thymus MHC molecules in epithelial cells in order to rescue these cells form programmed cell death. Binding to class I or class II MHC molecules instructs the cell to switch off expression of the CD4 and CD8 genes, respectively, and to assume the pre-T killer and pre-T helper phenotype, respectively. These differentiation events are accompanied by an increase in αβ TCR^- CD3 complex expression on the cell surface and occur in noncycling thymocytes. If the receptor binds to the specific peptide plus the MHC molecule, premature cell death is induced.

Mature T cells can expand considerably in secondary lymphoid organs. This depends entirely on the binding of the TCR to specific peptides and MHC molecules. If specific peptides are continuously present and at a high concentration, the cells will stop to divide and disappear from lymphoid tissue. Some of the cells will, however, remain and become refractory to antigenic stimulation which is accompanied by down regulation of TCR and co-receptors. Thus, with regard to specificity and mechanism, clonal selection in primary lymphoid organs is entirely different from clonal selection in secondary lymphoid organs.

Developmental Switches of Gene Control

B 012 REGULATION OF GLOBIN GENE EXPRESSION DURING ERYTHROID DEVELOPMENT, Gary Felsenfeld, Stephen Clark, Todd Evans, Gretchen Gibney, Joseph Knezetic, Catherine Lewis, Mark Minie, Joanne Nickol, Marc Reitman, Cecelia Trainor and Heiner Westphal. Laboratory of Molecular Biology, NIDDK, and Laboratory of Mammalian Genes and Development, NICHD, NIH Bethesda, MD 20892

The globin gene family has provided a popular model system for the study of developmental regulation of gene expression. We have carried out investigations of the organization of promoters and enhancers that control the globin genes of chicken, and have identified a variety of factors, some ubiquitous and some erythroid - specific, that bind selectively to the DNA of these regulatory regions. Some of these factors are present at all stages of erythroid development, and others are modulated in a way that is consistent with a stage-specific role in regulation. Among these factors are GATA1 (Eryf1), which plays a role in the regulation of all members of the globin gene family at every stage of erythroid development, and the PAL protein, which is a stage-specific member of the NF1 family associated with events occurring during terminal differentiation. It is not clear how these factors and their binding sites interact with chromatin structure to establish and maintain a transcriptionally active domain. However, we have carried out studies in transgenic mice which show that cis-acting elements in or near the centrally located β^A/ϵ enhancer can serve to confer position independence of expression of the β^A-globin gene when it is inserted into the genome. These results suggest that some of these elements must be capable, directly or indirectly, of providing signals for the disruption of the 30 nm chromatin fiber.

B 013 REGULATION OF THE HUMAN ß-GLOBIN GENE, F. Grosveld, M. Antoniou, E. deBoer, N. Dillon, P. Fraser, D. Greaves, O. Hanscombe, J. Hurst, M. Lindenbaum, S. Philipsen, S. Pruzina, D. Talbot and D. Whyatt, Laboratory of Gene Structure & Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

The human ß-globin gene family is located on the short arm of chromosome 11 and contains five functional genes. These are arranged in the same order as they are expressed during development, i.e. 5'-ϵ-γ_G-γ_A-δ-ß-3' over a distance of 55kb. The embryonic ϵ-globin gene is active when the yolk sac is the hematopoietic tissue, the γ-globin genes are active in the liver during the foetal stage, and the δ- and ß-globin genes in the adult stage bone marrow (for review, see Collins & Weissman, 1984). Each gene contains a number of tissue- and developmental stage-specific regulatory regions and the entire locus is controlled by the so-called Dominant Control Region (DCR). This DCR consists of four strong hypersensitive regions (HSS) upstream of the ϵ-globin gene. Addition of these regions confers copy number dependent expression on the human ß-globin gene in murine erythroleukaemia cells and transgenic mice, at levels comparable to the endogenous mouse globin genes. We describe a deletional analysis of three of these hypersensitive regions and show that 200-300bp fragments are sufficient to direct copy number dependent, integration site independent expression of the human ß-globin gene. Biochemical analysis in vitro and mutagenesis experiments in vivo show at least two erythroid specific proteins (NF-E1 and NF-E2) and one non- erythroid protein to be essential for the function of this region. Addition of the DCR to globin genes also results in altered developmental expression patterns of an individual gene. By using multiple globin genes, we show that the combination and order of genes is important for their expression. A model for the regulation of this multigene locus will be presented.

B 014 IMPLICATIONS OF THE CHROMATIN LOOP DOMAIN ORGANIZATION FOR STABLE GENE TRANSFER, A.E. Sippel, H. Saueressig, D.M. Winter and C. Bonifer, Institut fuer Biologie III, Schaenzlestr.1, D-7800 Freiburg/Br., Germany.

Nuclear DNA is organized in topologically constrained loop domains defining basic units of higher-order chromatin structure. In order to investigate the relevance of loop domains for the control of gene activity, we mapped a specific eukaryotic gene locus in respect to its chromatin organization. The active chicken lysozyme gene is located within a 20 kb chromatin domain of elevated DNase sensitivity of DNA. Various sets of up to 9 DNase I hypersensitive sites (DHSs) within this chromatin region correlate with the different regulatory transcriptional states of the gene. DNA transfection studies show that DHSs mark the positions of multifactorial regulatory elements (enhancers, silencer, steroid response element), each responsible for a specific sub-aspect of the total control of lysozyme gene activity. The domain of general DNase sensitivity terminates at both ends in specific DNA elements. The border elements are attached to nuclear matrix/scaffold material (MARs/SARs) and have a dual cis-activity (A-elements) for the function of genes when they are inserted into the genome. First, A-elements significantly elevate reporter gene transcription in the presence of enhancers and secondly, they buffer inserted "mini-domains" constructs from the regulatory influence of random neighbouring genomic regions (1). DNA transfer of entire genomic domains into cells in culture or into transgenic animals mediate position independent and tissue specific high-level transcriptional activity of transgene constructs (2). The results support the conclusion that eukaryotic genomes are organized in successive chromatin loop domains confining regulatory units for the independent control of gene loci.

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Growth Control and Oncogenesis

B 015 CELLULAR PROTEINS THAT ARE TARGETS FOR TRANSFORMATION BY DNA TUMOR VIRUSES. Karen Buchkovich, Nicholas Dyson*, Barbara Faha*, Chris Herrmann, Qianjin Hu, Emma Lees*, Jacqueline Lees*, Carol McCall*, Steven Shiff*, Li-Kuo Su, Li-Huei Tsai*, and Ed Harlow*, Cold Spring Harbor Laboratory, P. O. Box 100, Cold Spring Harbor, NY 11724 and *Massachusetts General Hospital Cancer Center, Charlestown, MA 02129

The identification of most tumor suppressor genes has been made possible by the discovery of inactivating mutations in these genes that occur during tumor development. Since the inactivation of both alleles is a key feature in the genesis of certain tumors, it has been postulated that the protein products of tumor suppressor genes function in the negative regulation of cell proliferation. Recent work from a number of laboratories has shown that the transforming proteins of several small DNA tumor viruses form protein-protein complexes with the products of tumor suppressor genes. The best studied case involves the interaction of viral oncoproteins with the retinoblastoma protein, pRB. The retinoblastoma gene was originally identified and cloned through its association with childhood retinoblastoma. However, it also appears to be a key target for transformation by the E1A proteins of adenovirus, the large T antigens of polyomaviruses, and E7 proteins of papillomaviruses, all of which bind to pRB. Genetic studies of these three proteins have shown that any mutation that destroys binding to pRB also destroys the ability of these oncoproteins to transform cells, suggesting that interaction with pRB is a key event in viral transformation. Because the viral transforming genes act in a dominant manner to transform cells and the products of tumor suppressor genes appear to act to inhibit cell proliferation, it is thought that these viral proteins inactivate pRB by physical interaction, thus mimicking the loss of pRB in naturally occurring retinoblastomas.

In addition to interacting with pRB, the adenovirus E1A proteins also interact with other cellular proteins. At the present level of detection, ten cellular proteins are known to bind to E1A either directly or indirectly. Most of these proteins are known only by their relative molecular weights; however, two are now known by name. In addition to pRB, E1A recently has been shown to interact with the human cyclin A protein. Cyclin A is a regulatory subunit of the cell cycle-regulated kinase, *cdc2*. Very little is known about the function of the cyclin A protein or the other proteins that bind to E1A; however, we are beginning to understand the structure of the interaction sites with E1A. pRB, p107, and p130 all bind to E1A in a similar manner. By several criteria, the p107 and p130 have been shown to display other properties, besides their binding properties, in common with pRB and, therefore, must be considered as additional candidates for targets of DNA virus-mediated transformation.

Analysis of pRB regulation has suggested that it has an active role in inhibiting cell cycle progression in the G0 and/or G1 phase. This function of pRB appears to be controlled by phosphorylation. In addition, mapping studies have identified an independently folding domain of pRB that is the site of interaction with the adenovirus E1A proteins and the SV40 large T antigen. This pRB region is affected in 23 of 25 naturally occurring mutations of the retinoblastoma gene that have been characterized to date, suggesting that the DNA virus proteins bind to an important functional domain of pRB.

B 016 TRANSFORMING GROWTH FACTOR α MEDIATED ONCOGENESIS IN TRANSGENIC MICE, Glenn Merlino[1], Chamelli Jhappan[1], Cheryl Stahle[1], Hitoshi Takagi[1], Nelson Fausto[2], and Gilbert Smith[3]. [1]Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892; [2]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI 02912; [3]Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, MD 20892.

Transforming growth factor α (TGFα) stimulates cellular proliferation *in vitro* and has been implicated in the transformation of many types of cultured cells. The effects of TGFα are mediated through a cell surface glycoprotein, the epidermal growth factor receptor (EGFR). To determine the consequences of perturbing TGFα signal transduction, three lines of transgenic mice were generated that contain a fusion gene consisting of the mouse metallothionein 1 promoter and a human TGFα cDNA. Human TGFα was zinc-inducible, and expressed in approximately half of the mouse tissues tested, including liver, pancreas and mammary glands. The effects of TGFα overproduction in these transgenic mice were found to be pleiotropic and tissue specific. The liver of 75% of transgenic male mice over 10 months old possessed well-differentiated hepatocellular carcinomas, compared to about 6% in nontransgenic male controls. Human TGFα RNA was elevated in liver tumors relative to adjacent grossly normal liver tissue in the same animal. The morphogenetic penetration of epithelial duct cells into the stromal fat pad of transgenic mammary glands was greatly impeded relative to age-matched nontransgenic female control mice. In addition, the multiparous founder of one line developed multiple breast lesions, including hyperplasias, adenomas and adenocarcinomas. These abnormal mammary tissues also contained higher levels of human TGFα RNA than their normal counterparts. Finally, the transgenic pancreas exhibited a florid ductular metaplasia, accompanied by severe interstitial fibrosis. These lesions developed in the pancreas of virtually 100% of the transgenic animals. Analysis of these lines of transgenic mice demonstrates that TGFα can act *in vivo* as a mitogen and a morphogen, and can contribute to neoplastic transformation.

B 017 STUDYING ONCOGENE FUNCTION USING ES CELLS AND TRANSGENIC MICE, E.F. Wagner, Research Institute of Molecular Pathology (IMP), Dr. Bohr-Gasse 7, A-1030 Vienna, Austria.

We have been introducing oncogenes into embryos and embryonic stem (ES) 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 inducible or constitutive promoter elements as well as with alterations at the 3' end were used to generate transgenic mice and chimaeras from c-fos expressing ES cells. The expression of exogenous c-fos specifically affects bone development in both systems, despite efficient expression in other organs. High c-fos levels in organs such as spleen and thymus affect the differentiation and function of lymphoid cells.

In a different experimental approach aiming to assay for dominant action of oncogenes in development, retroviral vectors carrying the selectable neo-gene together with a viral oncogene such as Polyoma middle T antigen or v-src were used for infection of ES cells. Chimaeric embryos obtained by blastocyst injection of individual ES cell clones expressing mT were specifically arrested at mid-gestation when blood vessel formation was disrupted by multiple haemangiomas. These endothelial tumours were used to derive endothelioma cell lines which, following injection into mice, rats, chicks and quails, induced the formation of **host** derived haemangiomas. A possible mechanism of mT action in endothelial cells will be discussed.

Finally, an mT vector was used to generate transgenic mice; in one family sympathetic hyperplasia and neuroblastomas developed with 100% penetrance. The relevance of these mice as models for human neuroblastoma will be reported.

Germline Mutation of Proto-Oncogenes

B 018 INACTIVATION OF THE PIM-1 PROTO-ONCOGENE BY HOMOLOGOUS RECOMBINATION IN ES CELLS, A. Berns, P. Laird, H. te Riele, E. Robanus Maandag, A. Clark*, and M. Hooper*, Division of Molecular Genetics of the Netherlands Cancer Institute, Amsterdam, The Netherlands. *Department of Pathology, University of Edinburgh, Scotland.

The pim-1 gene has been identified as an oncogene which encodes a protein serine/threonine kinase. pim-1 is frequently activated in murine leukemia virus-induced lymphomas in mice and can synergize effectively with c-myc in lymphomagenesis. The pim-1 gene is predominantly expressed in hematopoietic tissues and gonads. To get insight in the normal function of pim-1, we have inactivated the pim-1 gene by homologous recombination in ES cells. We have studied the effects of the absence of pim-1 protein both in ES cells, which normally highly express the pim-1 gene, and in mice. Efficient consecutive inactivation of the two pim-1 alleles in ES cells was achieved by using different selectable markers. The expression of these markers was made dependent on the acquisition of transcriptional and translational start signals from host DNA. ES cells lacking pim-1 protein were indistinguishable from the parental ES cells, both in growth characteristics and in differentiation potential, suggesting that the high expression of pim-1 in ES cells does not fulfill an essential role in the propagation or differentiation of these cells in vitro. The pim-1 null allele was also introduced into mice. The effects of complete loss of function of pim-1 in homozygous pim-1 mutant mice will be discussed.

Disease Genetics

B 019 p53 GENE MUTATIONS IN HUMAN COLORECTAL TUMORIGENESIS,

Suzanne J. Baker[1], James K.V. Willson[2], Sanford Markowitz[2], Antonette C. Preisinger[1], and Bert Vogelstein[1], [1]The Oncology Center and the Program in Human Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21231 and [2]The Ireland Cancer Center, University Hospitals of Cleveland, Case Western Reserve University, Cleveland OH 44106

Mutation of one copy of the p53 gene and concomitant deletion of the second allele occur in over 75% of human colorectal carcinomas, suggesting that the wild-type p53 gene may normally function as a colonic tumor suppressor gene. This is further supported by *in vitro* studies. Transfection of the wild-type gene into human colorectal carcinoma cells suppressed their growth in culture by preventing DNA replication. Mutations in the p53 gene such as those found endogenously in the tumors, however, abrogated its suppressive activity. Furthermore, the growth of a cell line derived from a benign colon adenoma was not altered by transfection with a wild-type or a mutant p53 gene. Evaluation of allelic loss and p53 gene mutations in primary benign colon tumors showed that both genetic alterations occurred rarely in adenomas. It appeared that mutation of the p53 gene was rapidly followed by loss of the wild-type allele. Thus, the p53 gene usually remains wild-type throughout the progression from normal mucosa to adenoma development. At some point in tumorigenesis, however, expression of the wild-type p53 may become rate limiting for cell growth, perhaps because of other genetic alterations that have accumulated. Selection for p53 point mutations and allelic deletions might occur very rapidly at this point, and are likely to provide an important contribution to further tumor progression.

B 020 Structure of the WT1 gene and its inactivation in Wilms' tumors

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The WT1 gene encodes a zinc-finger polypeptide which is expressed in nephroblasts during a critical period in kidney development. The gene maps within the smallest region of overlap among deletions in chromosome band 11p13 which have been described in Wilms' tumors. The majority of Wilms' tumors, however, do not show gross DNA rearrangements and express high levels of WT1 mRNA. Nucleotide sequence analysis of WT1 transcripts shows one of eight sporadic Wilms' tumors to have a mutation within the gene, resulting in loss of the third of the four zinc finger domains. In this case, inactivation of the WT1 gene is part of a series of events leading to the development of Wilms' tumor. The WT1 gene itself consists of 10 exons, spanning some 50 kb of genomic sequence. Normal kidney cells express two alternative splices in WT1, resulting in four distinct transcripts. The first alternative splice adds 17 amino acids, rich in serines and threonines, between the zinc finger domains and the amino terminus. The second splice adds 3 amino acids in the "knuckle" between zinc fingers 3 and 4. These alternative splices are highly conserved between the human and mouse WT1 genes, implying a functional significance.

B 021 TRANSGENIC MOUSE MODEL FOR HUMAN AUTOSOMAL DOMINANT DISEASE : FAMILIAL AMYLOIDOTIC POLYNEUROPATHY, Ken-ichi Yamamura, Shoji Wakasugi, Shigehiro Yi, Fumi Tashiro, Shuichiro Maeda and Kazunori Shimada, Institute for Medical Genetics, Department of Pathology and Dapartment of Biochemistry, Kumamoto University Medical School, Kumamoto 862, Japan

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder characterized by extracellular deposition of amyloid fibrils and by prominent pheripheral and autonomic nerve involvement. FAP is shown to be caused by the mutation of transthyretin (TTR) gene. In case of Japanese type the valine at position 30 was substituted by methionine (hMet30). All Japanese FAP patients so far examined are heterozygotes carrying one normal and one mutant gene suggesting that the main cause of FAP is the presence of hMet30. However, the big variation of the age at onset suggests the involvement of other factor in the development of FAP. The elucidation of this factor is very important in order to devise a new method of treatment. To analyze this factor we produced transgenic mice. The genes introduced are hMet30 gene carrying either its own promoter (0.6-hMet30) or mouse metallothionein promoter (Mt-hMet30), and human serum amyloid P component (hSAP) gene. First, we comfirmed the amyloid deposition and its progression with aging in transgenic mice carrying hMet30 gene. The presence of hMet30 as well as mouse SAP (mSAP) was shown by immunohistochemical analyses suggesting that the mSAP plays a same pathological role in the process of amyloid deposition as hSAP. Then,we found that the amyloid deposition can occur in one 1 year-old but not in other transgenic mouse although the serum levels of hMet30 are the same in these transgneic mice. This result clearly suggests that the amyloid deposition itself starts later in life, and that several factors can affect this initiation of amyloid deposition. Findings obtained from analyses on single or double (hMet30 x hSAP) transgenic mice are as follows : (1) the tetramers composed of mostly hMet30 are important for deposition as amyloid due to physico-chemical property of this molecule, (2) hSAP does not affect the initiation and progression of amyloid deposition, (3) microenvironment in each tissue, such as blood flow and loose tissue structure, can affect greatly the amount of amyloid deposition, (4) purely environmental factor, for example living condition, can affect the amyloid deposition. Analysis on environmental factor is now in progress.

Gene Therapy

B 022 EFFICIENT GENE TRANSFER WITH HERPES SIMPLEX-BASED VECTORS, Johnson, P.A., Roemer, K.B., Yoshida, K., Gage, F.H., Roman, M., Miyanohara, A. and Friedmann, T. Departments of Neurosciences and Pediatrics/Molecular Genetics, UCSD School of Medicine, La Jolla, CA 92093-0634.

Retrovirus vectors have become useful and versatile tools for efficient transfer and stable expression of foreign genes in mammalian cells. Since susceptibility to retrovirus infection requires replicating cells, post-mitotic or quiescent cells such as most neurons, differentiated hepatocytes and myotubes are not suitable targets for retrovirus vector-mediated gene transfer.

Vectors based on herpes simplex virus type-1 (HSV) have been developed which are capable of infecting a variety of these post-mitotic cells. HSV-based vectors have no transforming functions, they do not integrate into the host cell genome and they have a large potential capacity for added sequences. We have prepared HSV-based vectors by recombining reporter genes into parent HSV genomes containing mutations in genes essential for lytic viral growth. Such vectors are highly efficient at introducing and expressing foreign genes such as the E.coli β-galactosidase gene into post-mitotic and quiescent cells, including a variety of neuronal cells, hepatocytes, and myotubes. However, mutant HSV vectors defective for lytic growth have, so far, been cytopathic to infected cells. Furthermore, we have observed that the expression of non-herpes regulatory sequences does not reflect their function in the context of the cellular genome or their expression from plasmid vectors. HSV-based vectors suitable for efficient and stable transduction of mammalian cells will become more widely useful as the mechanism underlying these phenomena become better understood.

B 023 HEPATOCYTE TRANSPLANTATION IN TRANSGENIC MICE AS A MODEL OF GENE THERAPY FOR HEPATIC DEFICIENCIES, Savio L.C. Woo, Ph.D. Howard Hughes Medical Institute, Department of Cell Biology and Institute of Molecular Genetics, Baylor College of Medicine. Houston, Texas 77030

There are dozens of known metabolic disorders secondary to hepatic deficiencies in man, which may be corrected by development of technologies to deliver the corresponding genes into the liver. We have previously reported the successful isolation of primary hepatocytes from mice and demonstrated that functional genes can be efficiently introduced into them using defective retroviruses as the vectors, and the transduced genes remain functional in the hepatocytes for weeks in culture. Similar experiments have been reported using primary hepatocytes isolated from rats, rabbits and dogs. The next critical step will be the transplantation of these primary hepatocytes into living animals and look for long-term survival in vivo as well as continued expression of the transduced genes. To avoid immune rejection of the transplanted cells in heterologous hosts, we isolated hepatocytes from transgenic mice expressing a marker protein in the liver which is secreted into blood. The hepatocytes were transplanted into congenic recipient mice by direct injection into the portal vein on the spleen, and the marker protein was detected in the plasma of the recipients by radioimmunoassay. The marker protein started to appear in the blood within days post transplantation, and the level remained stable for 9 months. The results conclusively demonstrated that transplanted hepatocytes not only can survive long term in the host, but also continue to express hepatic functions as the marker gene is transcribed from a liver specific promoter. To address the question of where the transplanted hepatocytes reside in the recipients, a second transgenic line expressing the E-coli β-galactosidase gene in the liver was created. After portal vein or intrasplenic transplantation, blue hepatocytes were found in the normal liver parenchyma of the recipients with X-gal staining. These results demonstrated that hepatocytes transplanted into the spleen can migrate to the liver, and suggest that somatic gene therapy for hepatic deficiencies may be feasible by hepatocyte transplantation after hepatic gene transfer.

Transgenes and Biotechnology

B 024 TARGETING GENE EXPRESSION TO THE MAMMARY GLAND, A.J. Clark, A.L. Archibald, S. Harris, M. McClenaghan, J.P. Simons, C. Watson and C.B.A. Whitelaw, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, Scotland.

We have been developing transgenic animals as production systems for foreign proteins in milk[1]. To target expression to the mammary gland DNA sequences from the milk protein gene β-lactoglobulin[2](BLG) have been incorporated into fusion genes and tested in transgenic mice. 4.3 kb of 5' flanking sequences from BLG are sufficient to drive efficient expression of a genomic mini-gene encoding human α1-antitrypsin. Levels as high as 8mg/ml of this protein are detected in the milk of transgenic mice and furthermore, the product is as biologically active as its natural (human plasma-derived) counterpart[3]. Resection analysis of the 5' flanking sequences shows that the essential regulatory elements are located in a short segment of DNA just upstream of the cap site. The chromatin configuration and the binding of nuclear factors to this region are described.

1. Clark et al., 1989. Bio/Technology 7, 487-492.
2. Simons et al., 1987. Nature 328, 530-532.
3. Archibald et al., 1990. Proc. Natl. Acad. Sci. 87, 5178-5182.

B 025 EXPRESSION OF HUMAN PROTEIN C IN THE MILK OF TRANSGENIC MICE, Janet M. Young, Da-Wei Zhang, William H. Velander*, John Johnson*, Francis C. Gwazdauskas*, Anuradha Subramanian*, Tulin Morcol*, Rodolfo Conseco-Sedano*, Raymond Page* and William N. Drohan, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855, Chemical Engineering Department, Virginia Polytechnic Institute, Blacksburg, VA 24061*

The inability to express high levels of certain biologically active proteins in animal cell production systems has made the transgenic animal a potential bioreactor model for over production of pharmaceutically important proteins. Mammary gland specific expression of plasma proteins is attractive in that proteins produced in the mammary gland are post translationally modified and expressed at high levels. Because the mouse whey acidic protein (WAP) is expressed at high levels during lactation, our laboratory is examining the ability of the WAP promoter to express high levels of human protein C in the milk of transgenic mice. Activated protein C is a protease which functions to modulate thrombotic potential by inactivation of factors VIIIa and Va and thus has therapeutic potential in clinical situations leading to hypercoagulation including sepsis, post-operative states, liver disease, and malignancies. WAP/protein C DNA constructs have been used to generate transgenic mice. One construct directs expression of protein C at levels ranging from 0.2 to 4 micrograms/ml in milk of founder animals as measured by ELISA and chromogenic amidolytic assays. Western analysis demonstrates that the transgenic hPC is a heterodimer consistent with the form seen in hPC derived from plasma and the protein appears to be glycosylated. Anti-coagulant activity in a prolonged clotting assay is also observed, demonstrating at least partial gamma-carboxylation.

B 026 REGULATION OF A MILK PROTEIN GENE, Lothar Hennighausen*, Thomas Burdon*, Robert McKnight*, Avi Shamay*, Lakshamanan Sankaran* and Robert Wall#, *Laboratory of Biochemistry and Metabolism, NIDDK, NIH, Bethesda, MD 20892, #Reproduction Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705.

We use the mouse whey acidic protein (WAP) gene as a model to study transduction signals and genetic elements which confer integrated spatial, temporal and hormonal regulation of milk protein genes. The WAP gene is expressed almost exclusively in the mammary gland, the steady-state level of its mRNA increases several thousand-fold during pregnancy and transcription is synergistically induced by steroid and peptide hormones.

Transgenic mice have been used to identify elements associated with the various levels of regulation of the WAP gene. The promoter/upstream region conferred tissue-specificity to heterologous genes, but failed to respond correctly to developmental and hormonal signals. A mouse WAP transgene encompassing the promoter/upstream region, the transcribed sequences and 3′ flanking DNA was expressed at comparable levels of its endogenous counterpart, indicating the presence of additional control sequences 3′ of the promoter. However, developmental and hormonal expression of this WAP transgene was highly dependent on the site of integration, suggesting that the transgene did not represent an independent transcription unit or chromatin domain. Furthermore, it appears from the transgenic studies that expression of the WAP gene during pregnancy and lactation is subject to different mechanisms. In search for additional developmental and hormonal control elements we analyzed larger genomic fragments in transgenic mice.

It has been proposed that matrix attachment regions (MARs) or A-elements can confer integration site independent and copy number dependent expression to stably integrated genes in tissue culture cells. To test the hypothesis that such elements can establish an autonomous chromatin domain in transgenic animals, we linked A-elements from the chicken lysozyme gene to the mouse WAP gene and analyzed expression in transgenic mice.

B 027 EFFECTS OF A hGH TRANSGENE ON MAMMARY DEVELOPMENT AND GLUCOSE REGULATION,
Majid Mehtali, Anne-Catherine Andres[1], Olfa Bchini, Brigitte Schubaur, Claudie Gautier, Marianne LeMeur, Pierre Gerlinger and Richard Lathe[2]. LGME-CNRS, 11 Rue Humann, 67085 Strasbourg, France, [1]Institute fuer Tumorforschung, Tiefenauspital, 3004 Bern, Switzerland, [2]AFRC Centre for Genome Research, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK.
We have constructed transgenic mice expressing hGH under the control of regulatory sequences derived from the mouse hydroxy-methylglutaryl coA reductase gene. Transgenic mice express hGH in all tissues, and levels of hGH in peripheral blood are in the range 0.1-4 ug/ml. Elevated IGF-1 transcript levels were observed, as expected, in the liver of transgenic mice. Precocious mammary gland development was observed in virgin transgenic females. WAP gene expression commences at 3 weeks of age and development correlates with levels of local hGH expression in the mammary gland. In explants from virgin control females prolactin failed to replace hGH in stimulating WAP expression, indicating that the effect of hGH is not mediated solely through an interaction with prolactin receptors. We have also observed that transgenic mice show altered glucose regulation. Our transgenic mice are maintained as systematic crosses between SJL and C57BL/6. Surprisingly, F1 hybrids of these two strains were found to be glucose-intolerant, while hGH transgenic animals of the same type were resistant. Analysis indicates that SJL mice harbor a dominant allele conferring glucose intolerance and ubiquitous hGH expression abrogates this intolerance. Elevated circulating IGF-1 levels may be responsible. Although IGF-1 is related to insulin, our data indicates that IGF-1 may act to stimulate glucose assimilation via IGF-1 binding to its own receptor rather than through cross-reaction with the insulin receptor.

B 028 PRODUCTION OF A FOREIGN MILK PROTEIN IN THE MAMMARY GLANDS OF TRANSGENIC PIGS.
R.J. Wall[1], V.G. Pursel[1], A. Shamay[2], R. McKnight[2], C. Pittius[2] and L. Hennighausen[2]. [1]Reproduction Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705; [2]Laboratory of Biochemistry and Metabolism, NIDDK, NIH, Bethesda, MD 20892.

Whey acidic protein (WAP) is an abundant milk protein in mice, rats and rabbits but is not present in milk of swine or other livestock. Its synthesis is confined to the functional mammary gland and is under the control of steroid and peptide hormones. To determine whether mammary gland regulatory elements from the WAP gene function across species boundaries and whether it is possible to qualitatively alter milk protein composition, we introduced the mouse WAP gene into the genome of swine. Approximately 200 copies of a 7.2 kb EcoRI genomic fragment containing 2.5 kb of 5′ flanking sequence were injected into 850 pig zygotes and 2-cell eggs. The injected embryos were transferred to 29 recipients of which 22 farrowed. Five out of 189 pigs born had incorporated WAP sequences. From those founders, three lines were established, two containing approximately 10 copies and the other 15 copies of WAP per cell. Milk and mammary tissues of lactating founders and/or offspring were analyzed. WAP-specific RNA was detected in mammary tissue biopsies of all three lines. In two lines the concentration of WAP RNA was equivalent to that of a 10-day lactating mouse, and the third line contained approximately 50% as much WAP RNA. WAP protein was detected in milk of all lactating females at concentrations between one to two grams per liter; these levels are similar to those found in mouse milk. To test tissue specificity of WAP gene expression, a pig from each of the two high expressing lines was slaughtered, and samples from 17 tissues were analyzed. In one line, WAP RNA was detected only in mammary tissue; in the other line, WAP RNA was also detected in salivary gland tissue at a concentration of approximately 1% of that measured in the mammary gland. Our results suggest that the molecular basis of mammary-specific gene expression is conserved between swine and mouse. In addition, the WAP gene must share, with other milk protein genes, elements which target gene expression to the mammary gland. Mouse WAP accounted for about 3% of the total milk proteins in transgenic pigs thus demonstrating that it is possible to produce high levels of a foreign protein in milk of farm animals.

Late Abstracts

GENE DISRUPTION AND SUBTLE MUTATIONS IN ES CELLS AND MICE, Allan Bradley, Ann Davis, Paul Hasty, and Ramiro-Ramirez Solis, Institute for Molecular Genetics, Baylor College of Medicine, One Baylor Plaza T932, Houston, Texas 77030

Gene targeting in ES cells is a powerful tool for generating mice will null alleles at many different loci. We have mutated a variety of proto-oncogenes and established these as null alleles in the mouse germ line. These mutations have very severe phenotypic consequences, consequently a full understanding of gene function will be facilitated by an allelic series of subtle mutations at these loci. To generate such mutations, we have developed a hit and run vector which generates point mutations at the target locus by a 2-step recombination procedure. The hit and run is both highly efficient and ensures that only the desired modification is made to the genome. The cell lines generated with the Hit and Run experiments are still capable of showing efficient chimaera formation despite extensive periods of *in-vitro* growth.

DIFFERENTIATION OF EMBRYONIC STEM (ES) CELLS TO HAEMATOPOIETIC STEM CELLS.

Juliana M. Chang[1], Li Chung-Leung[2], Greg R. Johnson[2] and R. Lindsay Williams[1*].

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The totipotent developmental potential of embryonic stem (ES) cells is being widely exploited as a vehicle for the introduction of specific genetic changes into mice. However the ability of these cells to differentiate into a wide variety of cells types under certain culture conditions in vitro suggests that ES cells may also be used as a tool to investigate further cell biological problems. Thus ES cells can be used as a source of early embryonic cells to study embryonic growth control and cell differentiation in culture away from the complications of studying development in utero (1). Furthermore the recorded ability of ES cells to form a variety of differentiated, mature cell types (1,2) raises the possibility that ES cell differentiation in culture may be directed so as to produce specific somatic stem cells. The availability of large quantities of defined somatic stem cells would allow analysis of otherwise rare cell types and may also provide a means of reconstituting defined cell lineages in vivo.

We have been investigating the differentiation of ES cells in vitro using the feeder-free MBL ES cell lines previously isolated and cultured in media containing recombinant leukaemia inhibitory factor (2,3). In one project we have been investigating the potential of ES cells to form haematopoietic cells in vitro. MBL-5 ES cells which are maintained in suspension culture in the absence of LIF form embryoid bodies of which the majority clearly contain erythroid cells. Colony assays of cells isolated from the embryoid bodies demonstrate the presence of both CFU-E and BFU-E haematopoietic progenitor cells in the embryoid bodies. Furthermore analysis of the contents of the blood islands has demonstrated the presence of mature haematopoietic cells from a number of lineages. To assay for haematopoietic precursor cells, irradiated syngeneic mice were injected with embryoid bodies. The presence of early haematopoietic precursor cells in the embryoid bodies was clearly demonstrated by the formation of ES-derived spleen colonies in the irradiated recipients. Furthermore preliminary analysis of long term reconstituted mice has demonstrated the presence of ES-derived haematopoietic cells. The contribution of ES-derived cells to the different haematopoietic cell lineages in long term reconstituted mice is currently being investigated to determine whether the ES cells have differentiated to form the pluripotent haematopoietic stem cells.

1) Doetschman, T., Eistetter, H., Katz, M., Schmidt, W. and Kemler, R. (1985) J. Emb. Exp. Morphol. 87, 27-45.
2) Williams, R. L., Hilton, D. J., Pease S., Willson, T. A., Stewart, C. L., et al (1988) Nature 336, 684-687.
3) Pease, S., Braghetta, P., Gearing, D., Grail, D. and Williams, R. L. (1990) Developmental Biology 141, in press.

Transgenes, Development and Disease

Recombination, Developmental Biology

B 100 INACTIVATION OF THE MOUSE MHC CLASS II $A\alpha^b$-GENE BY HOMOLOGOUS RECOMBINATION ,Horst Bluethmann, Frank Koentgen, and Michael Steinmetz F.Hoffmann-La Roche, Central Research Units, CH-4002 Basel, Switzerland

To design a new mouse model for investigating the role of MHC class II molecules in T-cell development and B-cell activation we used the recently established approach of targeted mutation in embryonic stem cells lines by homologous recombination. A genomic fragment of 4.0 kb in size containing the first two exons of the Aα-gene was cloned from a cosmid library. Into the first domain the neomycin resistance gene was inserted to disrupt the coding sequence of Aα. Using this construct as targeting vector, ES-D3 cells were transformed by electroporation. Neomycin resistant colonies were screened by PCR analysis for homologous recombination. A targeting index of 1 homologous recombination events per about 250 random integrations was obtained. Stem cells carrying a recombinated $A\alpha^b$ allele were injected into host blastocysts and reimplanted into foster mice to generate chimeras, which will be tested for germ line transmission of the disrupted gene.

B 101 INSERTIONAL MUTAGENESIS BY AN REV TRANSGENE IN THE CHICKEN, Michael J. Briskin[1]*, Rou-Yin Hsu[1], Joseph A. Schultz[2], Deborah Duricka[1], and Robert A. Bosselman[1], [1]Amgen Inc., Amgen Center, Thousand Oaks, CA 91320, [2]Arbor Acres Farm, Inc., Marlborough Road, Glastonbury, CT 06033

Transgenic chickens have been generated by infection of early embryos with a replication defective vector (ME111) derived from reticuloendotheliosis virus (REV). Twenty-one lines of transgenic chickens, each carrying a single provirus, were inbred to determine the phenotype of offspring homozygous for the transgene insertion site. One family (F8) yielded no offspring homozygous for the ME111 insert, indicating disruption of a locus essential for development. Inverse polymerase chain reaction was utilized to isolate a cellular sequence upstream of the proviral insert. Southern analysis of the pre-integration site revealed no detectable rearrangements apart from the ME111 insertion. Additionally, a sequence upstream of the insert has been isolated from a cosmid library and subsequently used as a hybridization probe in northern analysis. Several RNA species have been mapped within 10 kb of the proviral insert. We are currently probing RNAs from homozygous embryos to examine any alterations in these transcripts which may have been caused by the F8 proviral insertion.

B 102 CONSTRUCTION OF A VECTOR FOR HOMOLOGOUS RECOMBINATION, Shyam S. Chauhan, Susan E. Kane, and Michael M. Gottesman, Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Insertional inactivation of a gene is a powerful tool for defining its function. Homologous recombination between the cloned inactivated gene and its cellular counterpart allows the replacement of the cellular functional gene by its cloned inactivated copy. However, random integration events in mammalian cells after transfection are severalfold more frequent than homologous recombination events. Therefore, identifying the rare homologous recombination events in a vast pool of random integrations poses a serious problem. Recently, Capecchi and coworkers described a strategy for enriching the targeted events by the use of positive-negative selection (Capecchi, Science 244:1288, 1989). Based upon their strategy we have constructed a vector (pSSC8) using pGEM3 as the parental plasmid. pSSC8 contains two copies of the thymidine kinase (tk) gene on each side of a neomycin resistance gene (neo^r) with both tk and neo^r under the control of a strong tk promoter. The two tk genes are flanked by very rare restriction sites (Sfi I). There are unique multiple cloning sites located between the tk genes and the neo^r gene. Transfection of NIH 3T3 cells with this vector conferred resistance to G418 and sensitivity to gancyclovir. Gene targeting experiments using this vector are underway in our laboratory.

B 103 SRY IS A MEMBER OF A NEW FAMILY OF GENES IN MICE, J. Collignon, B. Capel, j. Gubbay, P. Koopman, and R. Lovell-Badge,Laboratory of Eukaryotic Molecular Genetics, MRC National Institute for Medical Research, The Ridgeway, Mill Hill,London NW7 1AA, UK.
A new candidate for the testis-determining gene (TDF in human,Tdy in mouse) has recently been described. This gene is called SRY (Sex-determining Region on the Y) in human and Sry in mouse. We have found that Sry is a member of a new family of embryonically expressed genes. In addition to Sry we have cloned four other genes of this family. These genes are characterised by the presence of a highly conserved putative DNA-binding domain which shares homology to HMG proteins class 1 and 2, human UBF and the Mc mating-type protein of S. pombe. Furthermore, two of these genes have features similar to a repressor like domain as defined in Krüppel, whereas another member of the family may contain an activator like domain. Progress in further characterization of this gene family will be presented.

B 104 DISRUPTION OF GENES IMPLICATED IN LYMPHOCYTE FUNCTION AND DIFFERENTIATION: THE TRANSCRIPTION FACTOR Oct-2 AND THE RECOMBINASE ACTIVATING GENES RAG-1 AND RAG-2, Lynn M. Corcoran, Tyler Jacks, Daniel Silver, Marjorie Oettinger, David Schatz and David Baltimore. Whitehead Institute of Biomedical Research, Cambridge, MA 02142.
We have identified several protein factors expressed predominantly in the B and T lymphocyte lineages that are important regulators of immunoglobulin (Ig) gene expression and rearrangement. Through the use of tissue culture lines representing several stages of differentiation, we now have some knowledge of the the contribution of individual factors and of the timing of each contribution to this complex process. Oct-2 is one of three or more B cell-specific proteins that, in combination with several ubiquitously expressed proteins, activate transcription of the Ig heavy chain enhancer and all Ig gene promoters. It is present at all stages of B cell differentiation and is required for high level transcription of Ig genes in B cells, but because of the large number of regulatory sequences and proteins present within this locus, the full range of influence of oct-2 from the earliest B cell precursor through to the plasma cell is not known. To address this problem, we have disrupted the oct-2 gene in embryonal stem (ES) cells, and have generated chimeric mice on the way to creating a homozygous oct-2-deficient mouse. In this way, we hope to assess the role played by oct-2 in the development of the immune system, and to discover what role it may play in the developing nervous system, where the gene is also expressed.
With similar motives, we have embarked on a project to disrupt RAG-1 and RAG-2, two genes intimately involved in the DNA rearrangements necessary to join V-(D)-J and constant gene elements. Progress in these projects will be reported.

B 105 EXPRESSION OF TWO HUMAN HOMEOTIC GENES IN HUMAN HEMATOPOIETIC CELLS AND DEVELOPING TISSUES. Yashuhiro Deguchi, Cecil H. Fox, Anthony S. Fauci, and John H. Kehrl. Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD.
The homeobox is a 180 bp protein-coding sequence of DNA originally found in genes important in the regulation of embryonic development in Drosophila melanogaster. The homeobox is conserved evolutionarily and homeotic genes are likely to be important in both mammalian development and differentiation including the commitment of hematopoietic stem cells to particular cell lineages. We have isolated 2 previously undiscovered homeotic genes from a human B cell cDNA library (HB24 and HB9). The HB24 clones provided 2250 bp of DNA sequence information. There is a single methionine codon initiated open reading frame which is in frame with a homeobox and a CAX repeat. The predicted molecular weight of the HB24 protein is 51,659 daltons which is in good agreement with in vitro transcription and translation of the cDNA. The single HB9 clone, although not full length, has been sequenced and the predicted open reading frame is in frame with a homeobox. The homeobox domain of HB24 is very homologous to a diverged, tissue-specific homeobox gene of Drosophila (H2.0) while HB9 does not have a highly homologous counterpart. We examined the expression of HB9 and HB24 in various hematopoietic cells and cell lines by Northern blots and RNase protection assays. We demonstrated variable levels of expression of these genes in lymphocyte and monocyte cell lines. We also detected expression of these genes in lymphoid and hematopoietic tissues (lymph nodes, thymus, spleen, bone marrow, and fetal liver) using in situ hybridization. Experiments are in progress to precisely delineate which cell types in these tissues express HB9 or HB24. In addition, we have found that these genes are expressed in a limited number of developing tissues. Thus, our findings provide further evidence for the hypothesis that homeotic genes serve as controlling elements in adult hematopoietic cell functions in addition to their role in early development.

B 106 STUDIES ON THE EXPRESSION AND REGULATION OF *HOX-2.3* AND *HOX-2.4 IN VIVO* AND *IN VITRO*.

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***Hubrecht laboratory, Uppsalalaan 8, 3584CT Utrecht, The Netherlands; #Department of Molecular Biology, Pasteur Institute, Paris, France.**

Murine Hox genes are expressed in different but overlapping domains along the Antero-Posterior (AP) axis of the developing embryo, in ectoderm and mesoderm. Recent evidence supports the assumption that these genes are true homologs of their Drosophila counterparts and provide positional information to sets of cells along the embryonic AP axis during pattern formation. Comparative studies have revealed that the more 3' the position of a gene in its cluster, the more rostral the anterior boundary of its expression domain. Hox-2.4 and Hox-2.3 are respectively the second and third more 5' member of the Hox-2 family. Our experiments using E. coli lacZ as a reporter gene have revealed that the Hox-2.4/Hox-2.3 intergenic sequences contain a promoter, and are responsible for differential Hox-2.3 expression during embryogenesis: they specifically mediate lacZ expression in the Hox-2.3-positive intermediate mesoderm derivatives in transgenic mice (Hox-2.3Sm1.5/lacZ transgene). Up to now, it is not clear whether the regulatory region defined in these experiments is involved in tissue-specific Hox-2.3 expression (ureter cell lineage), or whether it has to do with the maintenance of position-signalling information along the Antero-Posterior axis in the intermediate mesoderm. Sequences responsible for Hox-2.3 expression in the CNS, PNS and somitic and lateral plate mesoderm derivatives, and potential control element(s) mediating the Anteroposterior Hox-2.3 transcript distribution in these tissues are thus located outside the Hox-2.4/Hox-2.3 intergenic region. The Hox-2.3SH10/lacZ fusion construct, extending up to 6 kb upstream of Hox-2.3 (thus including Hox-2.4), and ending up 430 bp 3' of the Hox-2.3 polyA gives rise to a ß-gal staining pattern very similar to the Hox-2.3 transcript distribution in neurectoderm (CNS & PNS) and mesoderm of mid-gestation embryos. On the basis of our data, we assume that distinct regulatory elements are used by different subsets of cells expressing Hox-2.3. Furthermore it appears that regulatory elements mediating expression of one Hox-2 member (Hox-2.3) extend into the upstream transcription unit (Hox-2.4).

B 107 INACTIVATION OF THE p53 TUMOR SUPPRESSOR GENE IN THE MOUSE GERM LINE, Lawrence A. Donehower[1], Janet S. Butel[1], and Allan Bradley[2], [1]Division of Molecular Virology and [2]Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030

Structural alterations of the p53 tumor suppressor gene have been associated with a wide array of human cancers. To examine the role of p53 in tumorigenesis and mammalian development, we have generated embryonic stem (ES) cell lines in which one of endogenous p53 alleles has been inactivated by neo^R gene insertion following homologous recombination. The gene targeting strategy utilized a murine p53 3.7 kb genomic construct flanked by an HSV TK gene and interrupted in exon 5 by a polyA- neo^R gene driven by a pol II promoter. Following gene transfer into ES cells and G418/FIAU selection, two clones were identified by PCR and Southern analysis which have the expected altered p53 gene structure. One of the heterozygous ES clones was injected into C57BL/6 recipient blastocysts and implanted into pseudopregnant C57BL/6 female mice. The resulting offspring were highly chimeric. The chimeric males are currently being bred to generate germ line heterozygotes. These heterozygotes will be examined for increased tumor susceptibility and will be bred to determine the effects of a p53 nullizygous state on embryonic development.

B 108 TRAPPED GENES EXPRESSED DIFFERENTIALLY DURING EARLY MOUSE DEVELOPMENT

Ernst-Martin Füchtbauer and Heiner Westphal, Laboratory of Mammalian Genes and Development, NICHD, NIH, Bethesda, MD 20892

By utilizing a gene trap vector initially described by Gossler et al. (Science 244:403), we have identified three out of 500 ES cell colonies which express LacZ in tissue culture. In 2 of the cell lines the beta-galactosidase activity is observed only in differentiating cells and is localized in the cytoplasm. In one line the beta-galactosidase activity is localized in or at the nucleus and is mainly observed in undifferentiated ES cells. Currently we are injecting these cells in blastocysts to search for the expression pattern in the developing mouse embryo and to produce chimeric mice which might transmit the insertional mutation to the germ line and thus produce mutant strains of mice.
EMF is supported by a fellowship of the Alexander von Humboldt Foundation, FRG.

B 109 *Sry*, A CANDIDATE FOR THE MOUSE TESTIS DETERMINING GENE, John Gubbay, Peter Koopman and Robin Lovell-Badge, Laboratory of Eukaryotic Molecular Genetics, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

The sex of a mammalian embyro is determined by the action of a gene on the Y chromosome, the Testis Determining Gene, known as TDF in humans and *Tdy* in mouse. We have identified and cloned a candidate gene for *Tdy* in mouse, known as *Sry*, by virtue of its homology to its human counterpart. *Sry* maps to the mouse Y chromosome and in particular to the Sxr' fragment, the smallest region of the mouse Y chromosome known to be sex determining. *Sry* was also found to be deleted in a line of sex reversed XY female mice which have been shown genetically to be mutant in *Tdy*. By characterising the limits of this deletion we hope to prove that *Sry* is necessary for sex determination. In order to show that *Sry* is also sufficient to cause the development of testes in XX embryos we are currently using genomic *Sry* clones to construct transgenic mice. These experiments will also help to define the regulatory sequences controlling *Sry* expression in the embryo.

B 110 THE ROLE OF M1/69 IN T-CELL DEVELOPMENT, Margaret Hough, Robert Kay, R. Keith Humphries, Terry Fox Laboratory, BC Cancer Agency, B.C. Canada

The monoclonal antibody, M1/69, identifies structurally heterogeneous forms of a murine hemopoietic cell surface antigen which is expressed during specific stages of differentiation. In T-cells, M1/69 expression is restricted to immature $CD4^-CD8^-$ and $CD4^+CD8^+$ thymocytes whereas mature $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes and peripheral T-cells do not express the antigen. B-cells also show a fluctuating pattern of M1/69 expression which is dependent on previous exposure to foreign antigens. Pre-B cells, unprimed B-cells and B-cells differentiating into antibody producing cells all express M1/69 whereas memory B-cells that have undergone Ig class switching are negative for M1/69. A cDNA encoding the M1/69 peptide was cloned from a hemopoietic progenitor cell line and isolated by immunoselection of transfected COS cells. Sequence and biochemical analysis revealed that the mature peptide is only 30 amino acids with much of its mass arising from cell type specific glycosylation. The human homolog, which we recently cloned, is remarkably similar to murine M1/69 in both structure and patterns of expression. To assess the role of M1/69 in T-and B-cell development, we are currently attempting to generate transgenic mice in which extinction of M1/69 expression is prevented. For these experiments, we have constructed an expression vector using both the lck and immunoglobulin enhancers and the T-cell receptor promoter to drive the mouse M1/69 cDNA.

B 111 TESTIS EXPRESSION OF THE X-LINKED AND AUTOSOMAL ISOFORMS OF THE MOUSE PDH E1α GENE. Rocco C. Iannello and Hans-Henrick M. Dahl, Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Melbourne, Australia 3052.

The pyruvate dehydrogenase (PDH) complex is an essential enzyme system involved in aerobic glucose metabolism. The importance of this enzyme complex is well illustrated where deficiencies in PDH activity have been reported. As such, a knowledge of the gene structure and the molecular mechanisms involved in the regulation and expression of the subunits which make up the PDH complex is essential. Previous work carried out in this lab resulted in the identification of two genes, one coding for the somatic form and the other coding for a testis form of the human PDH E1α subunit. More recently, the genes coding for these isoforms in the mouse have also been identified. As in the human, the mouse somatic isoform maps to the X-chromosome whereas the testis specific isoforms in human and mouse map to chromosome 4 and 19 respectively. As part of an on-going analysis of the structural organization and the developmental regulation of this subunit we have initiated a study to investigate the expression of both the X-linked and autosomal form of the PDH E1α subunit in developing mouse testis. Using conventional Northern blot as well as PCR analysis, we have shown that while the X-linked isoform in new born mice testis is the major species, the autosomal gene is abruptly turned on and is expressed as the predominant species by day 20. In addition, two testis-specific RNA transcripts with slightly different mobility properties on formaldehyde/agarose gels were identified. Both appear to be differentially expressed in a temporal fashion. The significance of these results with respect to spermatogenesis will be discused.

B 112 EXPRESSION OF HOMEOBOX CONTAINING GENES DURING EARLY XENOPUS DEVELOPMENT
Milan Jamrich, Marie - Luise Dicksen, and Susan Mackem, Laboratory of Molecular Pharmacology, CBER, FDA, Bethesda, MD. 20892

Using PCR based technology, we have isolated more than twenty different homeobox containing genes that are expressed during early Xenopus development. Three of them, Mix1, Xhox3, and Xhox7 have been previously isolated from Xenopus; others have not been previously described. Some of them are homologous in their homeobox region to genes described in other species such as distal-less, labial, and msh of Drosophila. Others do not show a high degree of homology to known homeobox genes. One of them contains a POU homeobox homologous to the murine OCT3 gene. Most of these genes are not expressed in eggs and are activated during the blastula or gastrula stage of development. At least some of them are also expressed in adults in a tissue specific manner.

B 113 A HOMEOBOX GENE PROMOTER-LACZ TRANSGENE EXPRESSED AS A GRADIENT IN DEVELOPING MOUSE LIMBS
Beatrice G. Jegalian, Martin Blum and Eddy M. De Robertis,
Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024-1737

We fused a 5.2 kb fragment containing Promoter II of the human homeobox gene Hox 3.3 with LacZ and analyzed the resulting transgenic mice. The construct contains 3.8 kb of sequence upstream of the transcription initiation site, the first intron, and coding sequence up to part of the homeobox fused to lac Z. The endogenous Hox 3.3 gene is expressed in part of the spinal cord and spinal ganglia, somites 9-16, as well as some internal organs such as mesonephros and intestine, and in the limb bud. The transgene expression has the same anterior border as the endogenous gene for spinal cord, neural crest and somites. At the posterior end, there is considerable variability between different transgenic lines.

Most importantly, the transgene reproduces the normal gradient of expression in the limb bud, with maximal expression anteriorly and proximally (Oliver et al, 1988, Cell 55, 1017-1024). This should facilitate studying the mechanism of how a gradient of nuclear protein can be formed in an apparently homogeneous group of mesodermal cells. In addition, this promoter should permit testing the effect of misexpressing other homeobox genes in the region of the Hox 3.3 forelimb gradient.

B 114 GENE TARGETING IN MICE AS A TOOL TO STUDY ANTIGEN PRESENTATION, MoncefJendoubi and Eric O. Long, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

T cells can only recognize antigen when it is presented in the context of a molecule encoded by the major histocompatibility complex (MHC). In addition, antigen must be processed in order to bind to MHC molecules. The processing requirements for antigen presentation mediated by class I or class II MHC molecules differ. Class I MHC molecules generally present antigens that were processed endogenously, whereas class II molecules present primarily exogenous antigens. It has been postulated that the basis for this distinction was the presence of the invariant chain associated tightly with the class II $\alpha\beta$ dimers from the time of their biosynthesis in the endoplasmic reticulum until this trimeric complex reaches an endosomal compartment. According to this hypothesis, class II molecules expressed in the absence of the invariant chain would behave like class I and would present endogenous antigens. This, in turn, may have profound effects on the selection of T cells in the thymus. To test this, we are attempting to generate mice deficient in the invariant chain gene. A genomic fragment of the invariant chain gene, containing the neomycin-resistance gene in exon 2 and copies of the tk gene at both ends, has been introduced into embryonic stem cells. Putative homologous recombination events are being characterized prior to their injection into mouse blastocysts.

B 115 REGULATION OF EXPRESSION OF HOX 1.3, Corrinne Lobe and Peter Gruss, Dept. of Molecular Cell Biology, Max Planck Institute of Biophysical Chemistry, Göttingen, FRG.

The murine homeobox gene Hox 1.3 is expressed during embryogenesis and in embryo carcinoma (EC cells) differentiated with retinoic acid (Dony and Gruss (1987) EMBO J. 6:2965). Using DNase I hypersensitive site mapping, three upstream regions as well as a site within the intron were identified as putative transcriptional regulatory regions. Their activity as transcriptional regulatory elements has been analyzed by linking varying lengths of these sequences to the firefly luciferase coding sequence and using these constructs in expression assays in EC cells. The transcriptional elements were more precisely defined using gel shift assays with extracts from cell lines and embryonic tissues. A basal level of expression in cell lines requires 300 base pairs of upstream sequence, which includes constitutive elements at the primary transcription start together with a homeobox binding site (Odenwald et al (1989) Genes Dev. 3:158) and multiple GC boxes. The minimum promoter sequence required for expression in transgenic mice, however, has been shown to be 900 base pairs (Zakany et al (1988) Neuron 1:679). Within these additional base pairs required in the mouse there is an upstream enhancer corresponding to a DNase I hypersensitive site. This region has been implicated in the spinal cord-directed expression seen in these transgenic mice.

These elements cannot account for the correct Hox 1.3 expression pattern: In tissue culture cells, post-transcriptional mechanisms seem necessary to down-regulate expression in undifferentiated cells; In transgenic mice the coding sequence and upstream region extending to the Hox 1.2 homeobox yields only spinal cord expression. By continuing such expression and DNA-protein binding studies, the basis for the precise spatial and temporal pattern of expression of Hox 1.3 and other genes expressed during development will be established.

B 116 MOLECULAR CHARACTERIZATION OF THE STRUCTURE AND REGULATION OF THE MOUSE HOMEOBOX-CONTAINING GENES, *En-1* and *En-2*

Cairine Logan, Mark C. Hanks, Anna Auerbach, Wilson K. Khoo, Dhani Nallainathan, Nicholas J. Provart, and Alexandra L. Joyner; Samuel Lunenfeld Research Institute of Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5

The two mouse genes, *En-1* and *En-2* that share homology to *Drosophila engrailed*, are expressed early in development in a spatially restricted manner consistent with playing a role in pattern formation. To help identify conserved elements responsible for their function and pattern of expression, we have cloned the human and chicken *En* genes. A comparison of the amino acid sequence of all the *engrailed*-like genes reveals four highly conserved protein domains including the 60 amino acid homeobox. We have begun to look at the regulation of expression of the mouse *En* genes at the level of transcription using *lacZ* reporter gene constructs in embryonic stem cell derived chimeric embryos, transgenic mice and neuronal cell lines. In our first attempt, an *En-2* genomic fragment extending 2.5kb 5′ of the start of transcription, with or without the intron, was not sufficient to direct expression in an *En-2* specific manner in embryos, although in preliminary experiments both constructs were expressed at low levels in a cerebellar cell line. New reporter constructs are being made and tested that include the *En-1* or *En-2* transcribed sequences and adjacent 5′ and 3′ potential regulatory sequences with *lacZ* fused in-frame with the protein coding sequences. These experiments are an important first step in determining the molecular basis for the spatial and temporal pattern of expression of the mouse *En-1* and *En-2* genes. (Funded by the MRC and NCI of Canada)

B 117 THE REGULATION OF MYELIN BASIC PROTEIN GENE EXPRESSION DURING MOUSE DEVELOPMENT, Peter M. Mathisen, Carol Readhead and Leroy Hood, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125

In the central nervous system (CNS), myelin basic protein (MBP) is synthesized by the oligodendrocyte and is concomitant to the CNS myelination. Developmentally, MBP mRNA is first detected 7 days postnatally, peaking at 18 days, and decreasing to steady-state levels by 90 days. While MBP is expressed in the peripheral nervous system (PNS), it is only 5-15% total myelin protein, as opposed to the CNS, where MBP is at 30-40%. A mouse mutation *shiverer*, a deletion of about 60% of the MBP gene, produces no detectable MBP and results in mice with a shivering gait and reduced life span. Interestingly, it is only the CNS myelin that is affected while the PNS myelin appears normal. Thus, MBP is a gene product whose precisely regulated pattern of expression results in a differentiated cell-type that provides a physiologically essential function in nervous system development. We are studying the control mechanisms of MBP gene expression by defining the *cis*-acting elements responsible for regulating MBP transcription. A series of transcriptional fusions containing sequential deletions of the MBP 5′ flanking sequences and the bacterial *lacZ* gene have been constructed and used to produce a number of transgenic mice. The promoter activity is being assayed by examining *lacZ* expression pattern by X-Gal staining. Such promoter deletion analysis with extended amounts of 5′ flanking sequence in transgenic mice offers the opportunity to delineate control elements that would go undiscovered in other types of transfection assays. We are now able to examine the spatial regulation of MBP expression: are the same *cis*-acting elements used for the entire nervous system or are there CNS and PNS specific elements? Within the CNS, are there variations in the *cis*-acting elements producing region-specific requirements for MBP gene expression?

B 118 EXPRESSION OF A MOUSE GENE CONTAINING A *PAIRED*-RELATED HOMEOBOX IN EMBRYOS AND IN EMBRYONAL CARCINOMA CELL LINES AND THEIR DERIVATIVES Frits Meijlink, Dirk-Jan Opstelten, Eric Kalkhoven, Fried Zwartkruis, and Lia de Laaf. Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584CT Utrecht, The Netherlands.

Structural similarities of developmental genes of insects and mammals indicate that at least some of the molecular interactions that have a role in regulation of embryogenesis have been conserved throughout evolution. Much work has focussed on the murine homologs of the *Drosophila* homeotic genes. In addition, several mouse genes structurally similar to *Drosophila* pair-rule or segment polarity genes have been isolated, including the *wnt*, *engrailed*, *evx*, and *Pax* families. We have studied the expression pattern during embryogenesis of a murine gene which contains a homeobox that resembles those of the *Drosophila Paired* and *Gooseberry* genes (pair-rule and segment polarity genes, respectively). It was originally identified by Kongsuwan and coworkers [EMBO J. 7,2131-2138(1988)] and tentatively named *S8*. In embryos *S8* is expressed in a complex pattern in mesenchyme of the head, pharyngeal arches, heart, limbs, developing backbone and tail. No expression was found in neural tissue or endoderm. The distribution of *S8* transcripts suggests involvement in local pattern formation rather than in setting up or maintaining the axes of the embryo. *S8* expression correlates in many instances with sites of prospective connective tissue and is found in several developmentally active regions in which epithelio-mesenchymal inductive interactions are known to have an important role, including the limb buds, the facial primordia, the hair follicles and dental papillae. Since we plan to dissect the molecular mechanisms responsible for establishing the *S8* expression pattern, we have looked for signals that modulate the level of *S8* mRNA in cells cultured *in vitro*. We are currently analyzing the influence of growth factors and retinoic acid on *S8* expression in various cell lines including embryonal carcinoma cells and their derivatives and on embryonic stem cells.

B 119 CLONING OF MOUSE-AMH (ANTI-MULLERIAN HORMONE) AND STUDY OF ITS EXPRESSION IN THE DEVELOPING EMBRYO, Andrea Münsterberg and Robin Lovell-Badge, Laboratory of Eukaryotic Molecular Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, Great Britain

Anti-Müllerian Hormone (*AMH*), otherwise known as Müllerian Inhibiting Substance (*MIS*), is a member of the *TGF-β* family of growth factors. In male embryos it is responsible for the regression of the Müllerian duct system, which would in females give rise to the oviducts, uterus and upper vagina. AMH is also implicated in aspects of testis differentiation through the study of its effects *in vitro* and recently in transgenic mice. The bovine and human *AMH* genes were cloned a few years ago. However, in order to more fully understand *AMH* function and regulation it was essential to isolate the mouse gene. A number of cDNA clones have been isolated from a 14.5 dpc fetal testes library, using partial cDNA clones from bovine, human and rat *AMH* as probes. The mouse cDNA clones have in turn been used to derive probes for screening mouse genomic libraries and a number of clones have been isolated and characterised by sequencing. The pattern of *AMH* expression has been established by during embryonic and newborn development in male and female mice using *in situ* hybridisation. It is possible, that AMH is regulated by the product of the testis determining gene (*Tdy*), for which a candidate has recently been described and termed *Sry*. *Sry* is characterized by the presence of a putative DNA binding domain. *In situ* hybridisation has been used to look more precisely at the distribution of both *Sry* and *AMH* transcripts and the possible interaction of the two genes will be discussed.

B 120 INDUCIBLE EXPRESSION OF HOX 1.3 HOMEODOMAIN PROTEIN IN TRANSGENIC MICE Ward F. Odenwald[1], Ronald J. DeSanto[2], Shang-Ding Zhang[1], Peter Vos[1], and Heinz Arnheiter[2], [1]Laboratory of Neurochemistry, and [2]Laboratory of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, Maryland 20892/USA

To test the function of the murine *Antennapedia* class homeodomain protein Hox 1.3 *in vivo*, we have generated a line of transgenic mice in which the levels of Hox 1.3 protein can be controlled by injecting interferon. These mice contain in their genome multiple copies of a Hox 1.3 cDNA linked to 1.8 kb of the mouse Mx1 promoter, an interferon α/β inducible promoter that has been used previously in mice for ubiquitous, inducible expression of an antiviral protein (Arnheiter et al., Cell 62, 51-61,1990). Baseline Hox 1.3 transgene expression appears to be extremely low in many organs of these mice, consistent with the observation that they are healthy. However, spontaneous transgene expression is detected in a subset of peritoneal macrophages. When 40 μg of an *E. coli*-derived, hybrid recombinant interferon (specific activity on mouse cells: 2.5x107 U/mg of protein) is injected intravenously into adult mice, transgenic Hox 1.3 RNA accumulates in many organs rapidly and to high levels. The transgenic protein accumulates in the nuclei of both parenchymal and non-parenchymal cells as evidenced by labeling of cryostat sections of liver tissue with a Hox 1.3-specific rabbit antibody. Gel shift assays indicate that the induced transgenic Hox 1.3 protein binds to a Hox 1.3 DNA binding site. By injecting interferon into pregnant females, transgene induction can be detected in fetuses between day 11.5 of gestation and birth by Northern analysis of total RNA. We expect that these mice and six additional transgenic lines will become invaluable tools to probe the biofunction of Hox 1.3 during development and in adulthood.

B 121 CONDITIONAL SECRETION OF ANTIGEN: A NEW METHOD FOR THE SIB-SELECTION OF HOMOLOGOUS RECOMBINANTS, Andrew C.G. Porter and Jane E.Itzhaki, Department of Biochemistry, Oxford University, UK.

Several methods are now available for the selection or detection of rare cells that have undergone homologous recombination (HR) between a target chromosomal sequence and a transfected construct. A choice of methods is particularly important for genetic analysis and therapy in humans, where the use of germ cells is not an option, since somatic cells will usually require two rounds of targeting, one for each allele. Using the interferon (IFN)-inducible human gene 6-16 of HeLa cells as our target we have developed a method for detecting rare recombinants in pools of stable transfectants by assaying for the IFN-dependent secretion of human growth hormone (hGH) into the growth medium. Our targeting construct includes a portion of the target gene upstream of the gene for hGH. Also included is a constitutively expressed *neo* gene allowing for the selection of stable transfectants. The hGH and target gene sequences are chosen so that hGH gene expression is only possible if integration involves HR with the target gene or non-HR next to an arbitrary promoter. In the latter case hGH secretion is likely to be constitutive while in the former it will be IFN-dependent. We have screened a total of 6500 G418-resistant colonies in pools of 30-60 colonies and have found at least 8 pools to show inducible hGH secretion. IFN-responsive clones have been isolated from 2 of the latter pools by sib-selection and subjected to further analyses. In our system the method compares well with other methods such as conditional positive selection (using *gpt* and resistance to mycophenolic acid), positive and negative selection (for which we obtained only a ~2-fold enrichment over positive selection alone) and screening by PCR (which we found to be prone to false positives not caused by contamination).

B 122 PRODUCTION OF EMBRYONIC STEM CELL LINES DEFICIENT FOR THE CD2 GENE, Reimann, A. and K. Eichmann, Max-Planck-Institut für Immunbiologie, Freiburg

CD2 is a membrane glycoprotein expressed early in thymocyte development on virtually all cells of the T lymphocyte lineage. CD2 appears to have a role in cell adhesion and in transmembrane signalling by the alternative pathway, but the physiological implications of CD2 mediated adhesion and activation in vivo are entirely unknown. In order to assess the functional role of CD2 in vivo, mice deficient for this molecule would be of great value. To this end we have started to inactivate the CD2 gene in embryonic stem (ES) cells by homologous recombination. Generation of ß2 microglobulin (ß2M) deficient mice by this approach has already been reported. We attempt to repeat this successful experiment to monitor the quality of our technology. Targeting vectors including the neoresistance and HSVTK genes were constructed from the genomic CD2 and ß2M genes. $2x10^7$ ES cells were transfected by electroporation with 10ug of DNA of each vector. The ES cell transfectants surviving selection by both G418 and GANC were analysed for homologous recombination events by PCR. Of $2x10^7$ electroporated cells approximately $1x10^3$ G418 resistant cells were obtained as calculated by extrapolation. Approximately 0,15% of these cells survived GANC treatment, amounting to 150 cells out of the initially electroporated $2x10^7$. In each of 2 successful experiments, 3 independent clones could be identified which were mutated for either CD2 or ß2M, respectively. This amounts to 2% of the cells resistant to both Neo and GANC or 1 in $7x10^6$ originally transfected cells.

B 123 **TARGETING OF THE GENE ENCODING THE PERIPHERAL MYELIN PROTEIN P0 IN MOUSE EMBRYONIC STEM CELLS,** Norbert Stahl and Brian Popko, Department of Biochemistry and Biophysics, and Brain and Development Research Center, CB #7250, University of North Carolina, Chapel Hill, North Carolina 27599

Myelin is a multilayered membrane sheath surrounding nerve axons in the central and peripheral nervous system (CNS and PNS). A set of membrane proteins is exclusively found in the myelin sheath and thought to facilitate the formation and compaction of the myelin structure. One of these myelin proteins, P0, is found only in the PNS where it comprises more than 50% of the myelin protein. P0 is a member of the immunoglobulin gene superfamily and thought to act by self adhesion. To better understand the function of P0 in the myelination process, we plan to disrupt the P0 gene by homologous recombination in mouse embryonic stem (ES) cells. A targeting vector was constructed containing P0 genomic sequences interrupted by a gene conferring neomycin resistance. Additionally, one HSV-tk gene was inserted on each side of the P0 sequence. After electroporation of $3.6x10^7$ ES cells, 44 colonies resistant to G418 and gancyclovir were obtained which are being examined for targeted P0 loci.

B 124 INSERTIONAL MUTAGENESIS OF EMBRYONIC STEM CELLS AND MOUSE GERM CELLS USING PROMOTER TRAP VECTORS, M. Stevens, E. Neilan, E. Weng, M. Sander, P. Brown, and G. Barsh, Departments of Pediatrics and Biochemistry, Stanford School of Medicine, Stanford, CA 94305-5428. We are developing a system to allow conditional disruption of genes with recessive phenotypes. DNA vectors that capture endogenous splice donor sites have been constructed using *neo* and *lacZ* as reporter genes. A multiple splice acceptor sequence from the MoMuLV envelope gene is followed by a translation initiation signal, a flexible peptide bridge, and coding sequences from *neo* or *lacZ*. Inducible promoters derived from the human and sheep metallothionein IIa genes have been placed upstream of the splice acceptor sequence in antisense orientation. "Captured" cell clones in which the DNA construct has inserted into an intron of an actively transcribed endogenous gene are first selected by G418 resistance or β-galactosidase staining. After removal of selective pressure and activation of the metallothionein promoter, an antisense transcript is generated against coding sequences of the endogenous gene. In certain cases, this antisense transcript will function in trans, thereby inhibiting expression of the endogenous gene from the uninterrupted homolog.

We eventually plan to use these promoter trap vectors as insertional mutagens in the whole animal. To explore the feasibility of testis-specific retrotransposon expression, the MoMuLV 5' LTR has been replaced with regulatory sequences from the mouse protamine-1 gene. In transgenic mice, transcription of the MoMuLV genome in the developing testis may lead to the acquisition of new retroviral insertions in spermatocytes. By combining this approach with promoter trap constructs, it may be possible to examine the phenotypic effects of recessive mutations in whole animals by altering only one of the two homologs.

B 125 HOMOLOGOUS RECOMBINATION AT c-fyn LOCUS OF MOUSE EMBRYONIC STEM CELLS WITH DIPHTHERIA TOXIN A FRAGMENT GENE IN NEGATIVE SELECTION, Keiichiro Yoshida*†Takeshi Yagi†, Yasuyo Shigetani†, Naoki Takeda†, Issei Mabuchi‡, Tadashi Yamamoto§, Yoji Ikawa† and Shinichi Aizawa†,*Central Res. Laboratory, AJINOMOTO Co. Inc. Suzuki-cho 1-1, Kawasaki, Kanagawa-210; †Laboratory of Molecular Oncology, Tsukuba Life Science Center, Physical and Chemical Research Institute(RIKEN), Koyadai 3-1-1, Tsukuba, Ibaraki-305; ‡Department of Biology, College of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo-153; and § Department of Oncology, The Institute of Medical Science, The University of Tokyo, Shirokanedai, minato-ku, Tokyo-108, Japan

In attempt to produce a mutant mouse with embryonic stem cells, the critical step is the efficient isolation of homologous recombinants; the frequency of the homologous recombination is usually low and the potency of the cells to differentiate into germ cells is unstable in culture. Here, we report a new and efficacious method for such isolation in which the diphtheria toxin A(DT-A) fragment gene was used to negatively select non-homologous recombinants. In contrast to the use of herpes simplex virus thymidine kinase gene, the selection can be made singly by G418 without using a drug such as gancyclovir, a nucleotide analog. At the c-fyn locus, the DT negative selection enriched the recombinants about 10 fold, and half of the cells integrating with the neomycin phosphotransferase gene were homologous recombinants.

Hemopoietic System

B 200 Lymphokine Gene Expression using Retroviral Vectors, Yawen L. Chiang[1], Stanley Friedman[1], Marlene Hammer[1], Sandra Yang[1], Kirsten Wadhams[1], Daniel Kuebbing[1], Paul Tolstoshev[1], and W. French Anderson[2], [1] Genetic Therapy, Inc., Gaithersburg, Maryland, and [2] Molecular Hematology Branch, NHLBI, NIH, Bethesda, Maryland.

Retroviral vectors containing a variety of human lymphokine genes have been constructed and evaluated *in vitro* for gene expression in mouse 3T3 fibroblasts and human Tumor Infiltrating Lymphocytes (TIL). The aim of these studies is to augment the efficacy of adoptive immunotherapy procedures such as TIL cell therapy by localized expression of selected lymphokines. Tumor Necrosis Factor (TNF) vectors, containing either the natural or a heterologous secretion sequence have been evaluated, and shown to produce substantial levels of human TNF in the target cells *in vitro*. TNF expression by gene transfer in TIL cells is an excellent candidate for clinical testing to augment TIL therapy for malignant melanoma patients, since TNF is very effective in treatment of tumors in mice, but systemic toxicity has limited its utility in man. Vectors containing the genes for human Interleukin 2, and for alpha-interferon have also been constructed, and expression of these genes is currently under evaluation.

B 201 REGULATION OF γδ T CELL ANTIGEN RECEPTOR TRANSGENES AND THE DEVELOPMENT OF T CELLS BEARING THE αβ ANTIGEN RECEPTOR, Alexander L. Dent and Stephen M. Hedrick, Department of Biology and the Cancer Center, University of California, San Diego, La Jolla, CA 92093

T cells bearing the αβ antigen receptor (αβ T cells) develop to varying degrees in transgenic mice carrying functionally rearranged γδ antigen receptor genes. Some transgenic founder lines present a severe defect in αβ T cell development, while other founder lines with the same transgenic constructs allow normal αβ T cell development. Part of the defect in αβ T cell development in the former transgenic animals involves not being able to turn off transcription of the transgenes, while in the latter animals, αβ T cells can down regulate the expression of both γ and δ transgenes. This result suggests that down regulation of γ and δ antigen receptor genes is an essential step for the development of αβ T cells. Transcriptional regulation of the γδ receptor transgenes appears to be affected by the integration site of the transgenes.

B 202 ENHANCER SEQUENCES LOCATED 3' OF THE MOUSE IMMUNOGLOBULIN LAMBDA LOCUS SPECIFY HIGH LEVEL EXPRESSION OF AN IMMUNOGLOBULIN LAMBDA GENE IN B CELLS OF TRANSGENIC MICE, Sarah Eccles, Nitza Sarner, Miguel Vidal, Angela Cox* and Frank Grosveld, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK and *Celltech Ltd., Slough, Berkshire SL1 4EN, UK.

Mouse immunoglobulin lambda (λ) light chain genes lack the lymphoid cell specific enhancer found in the J-C intron of the heavy and kappa light chain genes. We have identified elements responsible for λ gene regulation by cloning DNA sequences flanking the functionally rearranged λ_1 gene in J558L myeloma cells and testing their influence on expression of the λ_1 gene in myeloma cells and transgenic mice. Two elements have been identified, one 4-10kb and the other 30kb downstream of the λ_1 gene, which individually exhibit enhancer activity in myeloma cells. Co-injection of cosmids containing the λ_1 gene and both the proximal and distal enhancers into fertilized mouse eggs resulted in high level expression of the λ_1 transgene in B cells of transgenic mice. DNaseI hypersensitive site mapping in J558L nuclei revealed several strong hypersensitive sites in the region of the 30kb downstream enhancer and a further strong hypersensitive site 2.5kb upstream of the V region. The contribution of upstream sequences to the control of λ gene expression is now being investigated.

B 203 ANALYSIS OF T-CELL SUBSETS USING TRANSGENIC MICE WHICH SPECIFICALLY BLOCK THE MAJOR THYMIC T-CELL DIFFERENTIATION PATHWAY. Hermann Eibel, Frank Brombacher, Petra Fiedler and Georges Köhler, Max-Planck-Institute for Immunology, Stuebeweg 51, 7800 Freiburg, FRG.

T-cells can be grouped into into a minor population expressing TCR $\gamma\delta$ chains and into the main population expressing TCR $\alpha\beta$ chains. The latter subset develops in the thymus. Starting from the rather heterogenous population of $CD4^-8^-$ cells they become $CD4^+8^+$ immature thymocytes. These develop then further into mature $CD4^+8^-$ or $CD4^-8^+$ T-cells. The $CD4^-8^-$ subset contains besides of the precursors for $CD4^+8^+$ cells also mature T-cells expressing either TCR $\gamma\delta$ or TCR $\alpha\beta$ chains. So far, the origin of the latter subset remained a mystery. In order to dissect the differentiation pathways of these thymocytes we generated transgenic mice expressing an α-CD8 Ig μ heavy chain gene in all populations of T-cells. The expression of the transgene blocks specifically *in vivo* the main differentiation pathway of $\alpha\beta$ TCR^+ cells at the stage of $CD4^+8^+$ thymocytes. This differentiation block causes a 10–50 fold reduction of the immature $CD4^+8^+$ and of the mature $CD4^+8^-$ and $CD4^-8^+$ populations. It does not affect the maturation of $\gamma\delta$ TCR^+ cells. Their population develops normally and is highly enriched in the α-CD8 μ transgenic mice. The absence of $\alpha\beta$ TCR^+ $CD4^-8^-$ T-cells demonstrates, that these cells must have expressed CD8 during their maturation. Having shed some light on the mysterious origin of these cells we conclude that $\alpha\beta$ TCR^+ $CD4^-8^-$ T-cells develop from $CD4^+8^+$ thymocytes.

B 204 EVIDENCE FOR AN INHIBITION OF Mo-MuLV EXPRESSION IN PURIFIED MURINE HEMOPOIETIC STEM CELLS CULTURED *IN VITRO*.
Mark P.W. Einerhand, Trudy A. Bakx, Jan W.M. Visser and Dinko Valerio, Institute for Applied Radiobiology and Immunology TNO, section Gene Therapy P.O.Box 5815, 2280 HV, Rijswijk, the Netherlands.

Retroviral vectors are widely used for the efficient transfer of foreign sequences into a large variety of different cell types including hemopoietic stem cells (HSC). However, upon reconstitution of the hemopoietic system of lethally irradiated recipient with infected stem cells the retrovirally introduced sequences are often poorly expressed. Since primitive hemopoietic cell lines do not exhibit this phenomenon we investigated the expression of replication competent Mo-MuLV in purified primary HSC in culture.
Cells that were on average 100-fold enriched for day-12 CFU-S were cultured in the presence of IL-1a and IL-3. As a consequence the absolute number of cells increased during a culture period of 8 days. However, the number of primitive progenitors cells (day-12 CFU-S) decreased exponentially with time. Based on these observations and the fact that monocytes and granulocytes appeared late in culture we concluded that active hemopoiesis occurred leading to the accumulation of differentiated cells at later stages of the culturing period. Following infection with wild-type Mo-MuLV and bulk culture of the purified stem cells virus production per cell remained low for four days before it started to increase. This phenomenon can be explained by a differential expression level between more primitive and mature cell types as has been observed with replication defective Mo-MuLV derived vectors.
Clonal analysis of infected hemopoietic progenitor cells revealed that only a small percentage of the infected cells produced Mo-MuLV. These results give further evidence for the presence of an expression block in primary hemopoietic stem cells cultured and infected in vitro.

B 205 CHROMOSOMAL ORGANIZATION OF THE GLOBIN GENE DOMAIN, Donald E. Fleenor and Russel E. Kaufman, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

The beta globin gene domain contains multiple regulatory elements required for the correct developmental and tissue-specific expression of the individual globin genes. Transgenes are expressed at normal levels only when surrounded by DNA from the 5′ end of the domain which contains strong erythroid-specific DNase I hypersensitive sites. We have examined the DNase I hypersensitive site located 20 kb 3′ of the beta globin gene to determine its potential role in the regulation of the beta globin complex. Our sequence analysis reveals that the region is very A/T-rich (80% over 900 bp), contains multiple topoisomerase I and II recognition sites, as well as several consensus recognition sequences for GF-1 and AP-1 binding. Functional studies demonstrate that the region serves as a scaffold associated region in both erythroid and non-erythroid cell lines, while gel mobility shift analyses confirm interactions with GF-1 and AP-1. We have produced transgenic mice which carry the human beta globin gene flanked by this 3′ region. While similar constructions utilizing the 5′ DNase I sites are expressed, transgenes containing the 3′ site are not expressed in transgenic mice, suggesting that this region does not function as a locus control region. We hypothesize that this region may function to organize the domain by serving as a torsion absorber through its scaffold attachment points and topoisomerase sites to relieve supercoiling created during replication or transcription. Such a structure flanking the domain may also function by damping the effects of adjacent regulatory elements, perhaps limiting the activation potential of the 5′ LCR.

B 206 GENE THERAPY STUDIES FOR HAEMOPHILIA B

Ann Gerrard, Fiona Watt* and George G. Brownlee
Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, and
*ICRF Laboratories, Lincoln's Inn Fields, London, WC2A 3PX, UK.

Haemophilia B is an X-linked bleeding disorder caused by mutations in the gene for clotting factor IX. Treatment for the disease by gene therapy is envisaged to involve removing a tissue from a patient, introducing the factor IX gene *in vitro*, then reintroducing these cells into the patient to provide a continuous supply of clotting factor IX into the circulation.

Our previous gene therapy studies for haemophilia B have concentrated on the use of fibroblasts as potential target cells (Anson et al, Mol.Biol.Med, 1987, 4, 11-20). We are now investigating the use of human keratinocytes, testing whether they are able to synthesise and secrete active clotting factor IX. The human factor IX gene was introduced into keratinocytes using a retroviral vector, as this is currently the most efficient method of introducing genes into large numbers of cells for gene therapy. Transduced keratinocytes were found to secrete clotting factor IX at levels of 500 ng/10^6 cells/24 hrs in tissue culture. We are now testing the ability of these keratinocytes to produce and secrete factor IX after transplantation into nude mice, and whether any factor IX produced can reach the circulation.

B 207 REQUIREMENT OF THE MURINE ACCESSORY MOLECULE, Lgp55, FOR T CELL DIFFERENTIATION IN THE THYMUS. William T. Golde and Marcia McDuffie. Barbara Davis Center for Childhood Diabetes, Box B140, UCHSC, Denver, CO 80262

T cell differentiation in the thymus initiates with proliferation and expansion of the newly-immigrant precursor cells and the expression of cell surface molecules required for intercellular interactions. Maturation of the T cell is completed by the "selection" of clones that can recognize foreign antigen in association with self MHC molecules but are tolerant to self.The CD4, CD8, and T cell receptor proteins play a major role in selection and tolerance and a newly described murine accessory molecule, Lgp55, appears to function in the early stages of thymic development. Using an organ culture system of thymic lobes isolated from fetal mice at appropriate stages of gestation, we have demonstrated that blocking the adhesion function of Lgp55 eliminates the development of the $\gamma\delta$ subset of T cells and significantly reduces the $\alpha\beta$ cells. We have identified the stage of differentiation in which these thymocytes appear to be arrested. Presently, we are investigating the mechanism of this blocking effect to more clearly understand the role of Lgp55 in T cell development.

B 208 IN VITRO INDUCTION OF EMBRYONIC STEM CELLS TO DEVELOP INTO T- AND B-CELL PROGENITORS. Jose C. Gutierrez-Ramos and Ronald Palacios. Basel Institute for Immunology. Basel, Switzerland.

We have defined culture conditions that support in vitro the differentiation of the embryonic stem cell line D3 into T-cell progenitors, B-cell progenitors and myeloid cells as assessed, at various times of culture, by FACS with a panel of monoclonal antibodies against several surface markers (Thy1.2, Pgp1, B220, Joro37.5, IgM, F4/80 and Mac-1). We found that different bone marrow stromal and fetal liver stromal cell lines have distinct capacities of inducing differentiation of D3 stem cells into T- and B-cell progenitors ($Thy1^+Pgp1^+Joro37.5^+$ or $B220^+IgM^-$) and myeloid cells ($F4/80^+$ $Mac1^+$). S. uch functional properties of the different stromal cell lines can be distinctly modified by recombinant interleukins (IL3,IL4, IL5,IL6 and IL7) when added at different times of culture. Thus, we have been able to define a precise sequence of signals required to induce in vitro embryonic stem cells into T- and B-cell progenitors or myeloid cells. Under certain culture conditions, the D3 stem cells develop preferentially along a particular pathway of haemopoietic differentiation. Also, if the sequence of signals provided (by stromal cell lines and interleukins) is altered, the final products as well as the efficiency of the system dramatically change. We also shall present data concerning the functional potential of the lymphoid progenitors generated in vitro by the D3 ES cells.

B 209 **ALLELIC EXCLUSION IN TRANSGENIC MICE EXPRESSING A HEAVY CHAIN DISEASE (HCD) μ PROTEIN** A. Iglesias*, D. Corcos, D. Bucchini and J. Jami, Institut Jacques Monod, Université Paris 7, France and * Max-Planck-Institut für Immunbiologie, Freiburg, FRG

Transgenic mice expressing a human HCD-type μ gene that encodes for a truncated Cμ protein show allelic exclusion of endogenous murine IgM. As the HCD truncated μ proteins are unable to associate with light (L) chains, B lymphocytes in transgenic mice display transgenic Cμ proteins on the surface without associated L chains. Nonetheless, transgenic spleens contain abundant κ-mRNA of mature size and B cell hybridomas from transgenic mice produce murine L chains but not H chains. These findings indicate that the transgenic VDJ-less Cμ protein is able to support endogenous L gene rearrangement and to bring about B cell maturation. No signs of malignancy were found so far in the transgenic mouse population, thus suggesting that early expression of a HCD protein is not the only factor involved in disease development.

B 210 DEVELOPMENTAL EXPRESSION OF THE SHEEP β^c HEMOGLOBIN GENE IN TRANSGENIC MICE. Donna King, Steven G. Shapiro*, Robert J. Wall, Vernon G. Pursel, and Caird E. Rexroad. Reproduction Laboratory, U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD 20705 and *Zoology Department, University of Maryland, College Park, MD 20742

Sheep and goats are the only mammals that possess a beta globin gene that is expressed specifically during the juvenile period of development. This gene, termed β^c, is activated at birth in concert with the adult beta globin gene. It is turned off several months later when maturity is reached. If the animal is subjected to erythropoietic stress later in life, the β^c gene is reactivated. A line of transgenic mice carrying a single β^c gene cointegrated with the human beta globin Locus Activating Region was produced in an attempt to define the genetic elements controlling this unique developmental expression pattern. The endogenous mouse beta globin genes switch directly from embryonic to adult expression at day 12 of gestation. Therefore, mice lack a distinct juvenile compartment for the expression of globin genes and should not necessarily be expected to have the trans-acting factors capable of regulating the developmental timing of the sheep β^c gene. However, in the transgenic mouse line the gene was first activated, and expression also peaked, at day 12.5 of gestation. β^c mRNA was detectable in red blood cells at decreasing levels through 9 weeks of age. Older transgenic mice did not express the gene. These results demonstrate that the gene and its immediate flanking region contain sufficient information to establish an expression compartment analogous to the juvenile compartment that is coincident with the onset of adult expression in mice. These results also demonstrate the ability of the mouse to serve as an appropriate system for more detailed investigation of the special β^c regulatory elements.

B 211 **EFFICIENT INTRODUCTION OF EXOGENOUS GENES INTO THE GENOME OF PRIMARY LYMPHOID CELLS,** Ming-Ling Kuo, Yacov Ron and Joseph P. Dougherty, Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854

One way to study the role of immunoregulatory (or putative immunoregulatory) proteins in T cell development and activation would be to test the effect of constitutive expression of these proteins during development and activation. This could be done by introducing the immunoregulatory genes into early primary lymphoid cells using retrovirus vectors followed by transplantation into appropriate recipients and monitoring T cell development and functions. The genes would be expressed using vector signals that would insure constitutive expression. However, protocols must first be established for efficient gene transfer into precursor cells that give rise to all or part of the lymphoid system.

We have attempted gene transfer into $CD4^-$ $CD8^-$ thymocytes (DN thymocytes), an immature thymocyte population that contains cells capable of repopulating a thymus and giving rise to mature T cells. We prestimulated the cells for 18 hours with IL-7, so they would proliferate during the infection stage of the gene transfer protocol. We then added the proliferating DN thymocytes to a confluent lawn of irradiated, vector virus-producing helper cells for 18 hours. One to three days post-infection genomic DNA was isolated from the lymphocytes followed by Southern blot analysis to determine the efficiency of gene transfer. Our data indicates that we are able to introduce one to ten vector proviruses per cell, a very efficient gene transfer. We are now examining expression of the transferred genes and will be transplanting the infected cells into SCID mice in the near future.

B 212 **B CELL DEVELOPMENT IN SCID MICE WITH REARRANGED μ AND κ TRANSGENES.**
Lamers, M.C., Mossmann, H. and Koehler, G., Max-Planck-Institut fuer Immunbiologie, Stuebeweg 51, 7800 Freiburg, FRG.
Severe Combined Immune Deficiency mice lack mature cells of the B and T cell lineage, due to a defect in the process that leads to productive rearrangement of T cell receptor (TCR) and immunoglobulin (Ig) genes. As a result B and T cell development is arrested in an early pre-B and pre-T cell stage. This phenotype can be explained by either a lethal defect caused by the aberrant recombination process or by the absence of an Ig heavy chain or TCR chain protein. Introduction of a fully rearranged Ig heavy chain transgene on the SCID background would predict that in both scenarios the pre-B cell is rescued: through inhibition of endogenous Ig heavy chain rearrangement or through expression of μ chain protein. Indeed, introduction of μ, μ+κ or deletional variants of μ chains all rescued the pre-B cell stage to a varying extent, most likely correlated to their level of expression. The "rescued" transgenic pre-B cells on the SCID background could be identified as Lyb 2.1^{++}, HSA^{++}, $B220^{+}$ and $I\text{-}A^{+}$ cells. Mature B cells were rarely seen. 3 of 12 BM-derived hybridomes had lost both germline alleles; 4 of 12 lost transgenic copies. These results favour a role for the μ chain in rescuing the pre-B cell compartment in the SCID mouse.

B 213 **Ontogeny and Function of T-Lymphocytes in Mice without the Expression of the CD8 Gene**

Wai-Ping Fung-Leung, Maja Vollenweider, Marco Schilham, Kenji Kishihara, Amin Rahemtulla, Julia Potter and Tak W. Mak.

The Ontario Cancer Institute, and the Departments of Medical Biophysics and Immunology, University of Toronto, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada.

CD8 is a distinct cell surface marker for cytotoxic T cells and is absent in helper T cells. During T cell ontogeny in the thymus, both CD8 and CD4 are expressed by immature T cells. These double positive cells are believed to be necessary for the processes of positive and negative selection of the mature T cell repertoire. The mature T cells bearing CD8 mainly recognize foreign antigen in association with class I major histocompatibility complex. CD8 in the mouse is expressed either as a homodimer of Lyt2, or a heterodimer of Lyt2-Lyt3; the surface expression of either requires the presence of Lyt2 molecule. Mice without the expression of CD8 were obtained through germline transmission of embryonic stem cells in which the Lyt2 gene had been disrupted by homologous recombination. The phenotypic analysis and the functional studies of the T cells in these mice will be discussed.

B 214 THE CHROMATIN STRUCTURE OF THE BETA-2-MICROGLOBULIN GENE DURING DIFFERENTIATION
Donald B. Palmer and P. Julian Dyson. Transplantation Biology, Clinical Research Centre, Watford Road, Harrow, Middlesex. HA1 3UJ. U.K.

The expression of both class I (H-2) and Beta-2-microglobulin (β_2m) genes are developmentally regulated. While a number of regulatory elements have been defined in the upstream region of β_2m and class I genes, it is not clear that these can fully explain the complex developmental regulation of these genes. We, therefore, decided to look for novel regulatory elements of the β_2m gene by analysing DNAase I hypersensitive (DH) sites. Five DH sites have been found within the vicinity of the β_2m gene. One of these sites (DH1), located around the promotor region, may correlate with the transcriptional activity of β_2m, since it is diminished in both the undifferentiated embryonic stem cell line CCE and embryonic carcinoma cell line F9. The remaining DH sites (2-5) are located downstream from the β_2m gene. The most proximal downstream site (DH2) located 5.5 Kb from the last exon, appears only to be present in F9 and CCE cells, indicating that this site may be involved with the down regulation of β_2m. In addition, this site is markedly diminished in differentiated F9 cells. The third and fourth (DH3 and DH4) sites are present in all cell types examined so far. We are presently performing functional studies by linking these DH sites with a reporter gene.

B 215 HYPERSENSITIVE SITE 2 OF THE DOMINANT CONTROL REGION OF THE HUMAN β GLOBIN GENE CLUSTER, Sjaak Philipsen, Sara Pruzina, Dale Talbot and Frank Grosveld, Natl. Inst. Med. Res., The Ridgeway, Mill Hill, London NW7 1AA, UK

The Dominant Control Region (DCR) of the human β globin gene cluster consists of 4 hypersensitive sites (HSS) located between 45 and 65 kb upstream of the β globin gene. The DCR confers erythroid-specific, high levels of expression to a linked gene in murine erythroleukemia (MEL) cells and transgenic mice. Expression is integration-site independent and follows copy-number of the transgene. We have previously shown that the functional activity of HSS 2 of the DCR is retained on a 225 bp restriction fragment. We found 6 discrete footprints on this fragment: footprints 1, 3 and 5 represent bindingsites for the erythroid-specific protein NF-E1, while footprints 2, 4 and 6 are ubiquitous and characterized by G-rich motifs. We now have generated 5', 3' and internal deletions of these footprints to assess the importance of each individual element. We will present functional analysis of these deletions in MEL cells, and data on the proteins required for proper functioning of DCR-type elements.

B 216 STUDIES ON THE AUTOSOMAL CHRONIC GRANULOMATOUS DISEASE (A-CGD) p47 GENE; EXPRESSION AND p47 LOCALISATION.

A.Rodaway *I.C.R.F., London*, C.Casimir, A.W.Segal *University College, London University*; D. Bentley *I.C.R.F., London.*

CGD is an inherited syndrome characterised by extreme susceptibility to microbial infection. Most autosomally inherited cases of CGD result from the absence of a 47kDa protein component of the phagocyte NADPH-Oxidase system. We have cloned cDNA and genomic sequences coding for this factor and have raised an antiserum to it. These data have been used to detect mutations affecting the gene in patients. Studies using the antiserum have shown that in response to stimulation of the cell (*e.g.* phagocytosis) p47 translocates from the cytoplasm to the membrane of the phagocytic vesicle. The protein contains two copies of a motif, SH3, which may be responsible for this membrane association.

The gene is expressed in phagocytic and B-lymphoid cell types, is induced upon differentiation of HL60 cells and is transcriptionally regulated by Retinoic acid. We are investigating the promoter in order to elucidate the elements responsible for this pattern of expression

Preliminary experiments are under way attempting to complement the mutation by transfecting the gene into cell lines obtained from patients. Success in these experiments would further the long term aim of correcting the defect by somatic gene therapy.

B 217 HUMAN RHEUMATOID FACTOR EXPRESSION IN TRANSGENIC MICE. Helen Tighe, Rebecca Tucker, Jean Roudier, Frank R. Jirik, Pojen P. Chen, Thomas J. Kipps and Dennis A. Carson. Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0945.

Human rheumatoid factors are encoded by a unique set of germline variable region genes. These genes are expressed in early fetal development and by a significant proportion of normal adult B lymphocytes in the absence of serum autoantibody synthesis. Regulation of expression of these genes, however, may be aberrant in autoimmune diseases, such as rheumatoid arthritis, in which high levels of rheumatoid factor are produced and pathologic immune complexes of the autoantibody with its target antigen IgG are deposited in the joints. To understand the physiology and regulation of human rheumatoid factor gene expression, we have developed transgenic mice that express immunoglobulin heavy and light chain variable region genes encoding a human IgM rheumatoid factor. We find human rheumatoid factor expressed on substantial proportions of B cells of two founder lines AB8 and AB29, but not on T cells, fibroblasts, macrophages, neurons or kidney cells. The rheumatoid factor B cells are distributed throughout the lymphoid tissues. In addition, the sera of such animals contain rheumatoid factor at levels up to 35 μg/ml. Although expression of the transgenic antibody on 40% of splenic B cells from the AB29 mice and 10% of cells from AB8 mice does not reduce total serum levels of mouse Ig, expression of mouse surface immunoglobulin apparently is down-regulated with human rheumatoid factor being present on mouse lymphocytes which express either low or undetectable levels of mouse IgM. Interestingly, there appears to co-ordinate expression of the human light and heavy chain genes although these were inserted on different gene fragments. The low levels of mouse immunoglobulin present on a proportion of these cells could mean that mixed molecules of mouse and human immunoglobulin may be expressed in the transgenics. These experiments are the first to demonstrate the production of a human autoantibody in a transgenic system.

B 218 A HUMAN T CELL-SPECIFIC TRANSCRIPTION FACTOR (TCF-1α) IS A MEMBER OF THE HMG FAMILY OF DNA BINDING PROTEINS.

Marian L. Waterman and Katherine A. Jones, The Salk Institute, La Jolla, CA 92037

We have recently purified a T cell-specific transcription factor (TCF-1α) that binds to the T Cell Receptor α (TCR-α) and TCR-δ enhancers, to promoters of genes expressed exclusively in T cells (CD3 δ,γ) or lymphocytes ($p56^{lck}$), and to the HIV-1 promoter. The TCF-1α binding site in the TCRα enhancer was shown to be critical for T cell-specific enhancer activity *in vivo* and *in vitro*. Complete α-enhancer activity also requires adjacent sequences that contain consensus binding sites for the CREB and *ets-1* transcription factors. Using tryptic peptide amino acid sequence information from DNA affinity-purified TCF-1α preparations and PCR technology, we have cloned the gene encoding TCF-1α. Full length clones were isolated from a λ ZAP cDNA library derived from Jurkat cells by hybridization to a random-prime labelled PCR fragment. Sequence analysis of these clones identified a 1754 bp open reading frame encoding a 400 amino acid protein (predicted Mr: 44.2 kD). TCF-1α mRNAs (4.5 and 3kB) are detected in Jurkat and CEM T cells and are not found in the JY B cell line or U937 macrophage cell line, or in non-lymphoid cells (HeLa, NIH 3T3). Expression of TCF-1α in bacteria or in *in vitro* transcription-translation reactions revealed that the 400 amino acid encoded protein migrates as a 54 kD protein, nearly 10 kD larger than its calculated size but identical to that of purified native TCF-1α. Southwestern analysis of bacterial TCF-1α confirmed that the protein binds specifically to TCF-1α DNA binding sites and not to a mutant TCF-1α binding site. TCF-1α is not highly related to any protein listed in current databases, but does possess limited homology to high-mobility-group (HMG) chromosomal proteins. This region of homology is also found in hUBF, a human nucleolar RNA polymerase I transcription factor, and in the human sex-determining Y chromosome-specific protein, SRY. Thus the HMG box signifies a new class of DNA binding proteins, at least some of which function as eukaryotic transcription factors.

Growth Control, Oncogenesis

B 300 TARGETING EXPRESSION OF SV40 T-ANTIGEN TO THE RETINA OF TRANSGENIC MICE. **Muayyad R. Al-Ubaidi, Joe Hollyfield, Paul A. Overbeek and Wolfgang Baehr. Baylor College of Medicine, Houston, Texas.**

Transgenic mice harboring the SV40 T-antigen gene develop tumors at sites where expression is allowed by the heterologous promoter. In this study expression of SV40 T-antigen was targeted to the retinal rod photoreceptor cells via the use of the promoter for the mouse opsin gene. The opsin gene is exclusively expressed in the pineal and the retinal rod photoreceptor cells. Three transgenic lines were produced, one of which did not pass the transgene to the offspring. Northern analysis of the remaining two lines showed the expression of the transgene in the retina and the brain. Although brain tumors were observed in both lines, no retinal tumors were found. Instead, retinal photoreceptor cell death was apparent at early stages of the developement of the retina. The photoreceptor cell death may be the result of a direct interaction between T antigen and a cellular protein that is required at certain stages of retinal rod photoreceptor cell development. We cannot rule out, however, that cell necrosis intiates through an immune system mediated response. Since retinal photoreceptor cell death occurs in both lines, we conclude that it is independant of the site of integration of the transgene.

B 301 ACTIVATION OF NERVE GROWTH FACTOR IN HTLV-I-TAX TRANSGENIC MICE: POTENTIAL AUTOCRINE MECHANISM FOR TRANSFORMATION, **Jeffrey E. Green, Laboratory of Molecular Virology, National Cancer Institute, Bethesda, MD 20892**

Human T-cell leukemia virus, type 1 (HTLV-I), is a retrovirus etiologically associated with adult T-cell leukemia and a neurodegenerative disease, HTLV-I-associated myelopathy. HTLV-1-tax transgenic mice have previously been shown to develop neurofibromas as well as tumors of the adrenal medulla, iris and ciliary body. Further characterization of the neurofibromas, and cell lines derived from them, reveals that they express nerve growth factor (NGF) and nerve growth factor receptor (NGFR), suggesting a potential autocrine mechanism for transformation. Transient transfection assays demonstrate that tax trans-activates the NGF promoter but not the promoter for NGFR. A tax-responsive element in the NGF promoter has been identified. Activation of NGF by HTLV-I-tax suggests that HTLV-I associated myelopathy may be related to abnormal secretion of neurotrophic factors through expression of tax in perineural cells

B 302 TISSUE SPECIFIC EXPRESSION OF HPV-16 E6 AND E7 GENE PRODUCTS IN TRANSGENIC MICE LEADS TO DEVELOPMENTAL DEFECTS. Anne E. Griep[1,2], Renee Herber[1], Jan Heideman[2] and Paul F. Lambert[1], Department of Oncology[1] and Biotechnology Center[2], University of Wisconsin, Madison, Wisconsin 53706.

The human papillomavirus type-16 (HPV-16) is one of the papillomavirus genotypes which is frequently associated with cervical carcinoma. In cervical carcinoma tissue and in cell lines derived from cervical carcinoma, two translational open reading frames of the HPV-16 genome, E6 and E7, are found to be structurally intact and actively transcribed, implicating their gene products as agents in the development of this epithelial cancer. The E6 and E7 gene products have been characterized as immortalizing agents on the basis of *in vitro* assays in tissue culture cells. The E6 and E7 gene products are also known to interact with tumor suppressor gene products, p53 and retinoblastoma, respectively. To study the specific activities of the E6 and E7 gene products in a differentiating epithelial tissue *in vivo*, we created transgenic mice carrying a chimeric DNA fragment in which the E6 and E7 open reading frames are fused to the transcriptional regulatory signals for the αA crystallin gene. This tissue-specific promoter directs the expression of E6 and E7 specifically to the developing lens, since it is activated before terminal differentiation of the lens epithelial cells. Transgenic mouse lineages have been established from three founder animals. All transgenic mice developed bilateral microphthalmia which was overtly apparent from late embryonic stages onward. Histological examination of lenses from transgenic mice indicated abnormalities in fiber cell differentiation. These abnormalities are similar to those which developed when other immortalizing gene products were expressed in the developing lens. Biochemical analyses are in progress to correlate the observed histopathology with onset of transgene expression and to evaluate at the biochemical level the perturbation in lens cell differentiation. Our results indicate that the primary combined effect of the HPV-16 E6 and E7 gene products on differentiating epithelia is to inhibit normal differentiation. This property may relate to the etiologic role of these viral genes implicated in cervical carcinoma.

B 303 **DEVELOPMENT OF BRAIN TUMORS IN MICE TRANSGENIC FOR THE NEU ONCOGENE UNDER THE TRANSCRIPTIONAL CONTROL OF THE MYELIN BASIC PROTEIN GENE.** Carol Hayes, Donna Kelly, Yasuto Higashi, Shigeo Murayama, Masaki Ohno, Kinuko Suzuki, and Brian Popko. Brain and Development Research Center, University of North Carolina at Chapel Hill, CB# 7250, Chapel Hill, NC 27599-7250.
Myelin is the multilayered membrane sheath that surrounds nerve axons and facilitates the rapid conduction velocity of the nerve impulse. In the central nervous system (CNS) oligodendrocytes are the cells responsible for myelinating nerve axons, whereas Schwann cells myelinate the peripheral nervous system (PNS). The myelin basic protein (MBP) is a structural protein of myelin that is abundant in both PNS and CNS myelin. We have set out to transform myelinating cells in vivo utilizing a transgenic approach. The activated neu oncogene was placed under the control of the transcriptional regulatory region of the MBP gene. We have used this DNA construct to generate fourteen transgenic founder animals, ten of which have been bred to homozygosity. Several animals from these lines have developed brain tumors. We are presently characterizing the origin of these tumors, histologically, immunohistochemically, and with molecular techniques.

B 304 TRANSGENIC HEPATOCARCINOGENESIS IN THE RAT, Heideman, J., Su, Y., Hully, J. H., Moser, A., Griep, A., Neveu, M., and Pitot, H. C., McArdle Laboratory for Cancer Research and The Biotechnology Center, University of Wisconsin, Madison, WI 53706
Utilizing a DNA construct (*alb*-SV) (*Oncogene*, 4: 715, 1989) we have generated transgenic rats that spontaneously develop hepatic foci and neoplasms. The *alb*-SV construct was injected into pronuclei of rat embryos collected from superovulated prepubescent Sprague-Dawley female rats mated with males of the same strain. Standard microinjection technique was utilized; however, a more accurate and aggressive microinjection into rat embryo pronuclei was required. Consequently, survival of injected embryos was low. To ensure adequate litter size for normal parturition and lactation, noninjected embryos collected from females mated with Copenhagen (hooded coat color) males were added as carriers. Of 50 potential transgenic animals screened, four were shown to carry the transgene by PCR and/or Southern blot hybridization. Two of these animals, one female and one male, have generated offspring which also contain the transgene. One male died at two and one-half months of age with massive hepatic neoplasia involving virtually the entire organ. This animal appeared to carry at least six copies of the transgene within its DNA. In rats exhibiting only one copy of the transgene, focal lesions quite similar to altered hepatic foci develop in the liver with an occasional small hepatocellular carcinoma exhibiting histologic characteristics of hepatoblastoma. These lesions develop within two months of birth; however, such animals have now survived for 4 months or longer. Histochemical studies of the focal lesions show an absence of γ-glutamyltranspeptidase and the placental form of glutathione-S-transferase as well as canalicular ATPase, glucose-6-phosphatase, and connexin 32, while the normal-appearing liver expresses the last three genes. SV40 T antigen is expressed at relatively high levels in the focal lesions but not in the remaining hepatocytes. This transgenic model of hepatocarcinogenesis in the rat may offer considerable potential in studying the effect of environmental factors on genetically programmed carcinogenesis in this species.

B 305 CKII, A GROWTH-REGULATED PROTEIN KINASE: INFLUENCE OF PROLIFERATION-MODULATING AGENTS ON ITS ACTIVITY IN VIVO AND IN VITRO; Olaf-Georg Issinger and Brigitte Boldyreff, Institut für Humangenetik, Universität des Saarlandes, D-6650 Homburg, Germany
CKII, is a protein kinase which has been shown to be elevated in proliferating and differentiating cells, during certain stages of embryogenesis, and in solid tumours. It is found in the nucleolus and cytoplasm depending upon the cellular stage. It is a pleiotropic enzyme inasmuch that it phosphorylates numerous proteins. One of its best substrates is nucleolin, a 110 kDa protein which participates in rDNA transcription. We have used synchronized cell cultures to study the effect of exogenous agents, which have been shown to interfere with proliferation and differentiation processes in mammalian cells (e.g. TPA, retinoic acid, quercetin, indomethacin, 2.3-DPG, heparin, DFMO etc.). CKII has been shown to play an important role in rDNA transcription by phosphorylating nucleolin. Consequently we have investigated the influence the above mentioned agents on CKII activity vitro and in vivo. By using recombinant CKII alpha subunit (which is the catalytic subunit of the tetrameric holoenzyme) it was possible to study the site of interaction of some of these agents with CKII.

B 306 TRANSFORMATION BY SV40 t MUTANTS APPEARS RESTRICTED, *IN VIVO*, TO CYCLING CELLS, A. S. Levine, C. Cicala, F. Pompetti, and M. Carbone, NICHD, Bethesda, MD 20892.

The SV40 small t-antigen gene (t) performs an essential function in infection since it has been highly conserved, but its role in transformation is less clear. Studies in our lab indicate that deletions in t alter the oncogenicity of SV40. In contrast to wild type (wt) SV40, which only induces fibrosarcomas (in newborn hamsters) at the site of subcutaneous (SC) injection, the t mutants dl 883 (4532 - 4588 nt deleted), dl 884 (4502 - 4748) and dl 890 (4720 - 4746), injected SC, also induce abdominal lymphomas. The tumors comprise cells from a unique population of macrophages that retain the ability to divide despite a high degree of differentiation (MAC-2+). An additional mutant, dl 2006 (4560 - 4809), which contains the largest deletion in t, induces osteosarcomas when injected SC. To investigate the oncogenicity of these mutants under conditions which allow many different cell types to be exposed to high concentrations of the virus, we introduced SV40 virus by intracardiac (IC) injection. Groups of 12 Syrian hamsters (21 day-old) each were injected with $10^{8.5}$ pfu of dl 883, dl 2006 or wt 830 respectively. All but one of the animals developed tumors: dl 883 only induced lymphomas; 2006 mainly induced lymphomas and osteosarcomas (74%). In contrast, wt 830 induced lymphomas, osteosarcomas, and soft-tissue sarcomas, but 56% of the animals developed mesotheliomas, tumors not previously associated with DNA virus transformation. Tumors induced by t mutants were apparently all derived from cell populations that have, *in vivo*, a high proliferative rate (lymphoblasts, osteoblasts). Wt SV40 also transforms cells that normally do not have a rapid turnover, i.e., mesothelial cells. These results suggest that: 1) SV40 t mutants, *in vivo*, preferentially transform particular subtypes of rapidly proliferating cells, and 2) most wt SV40 tumors induced by IC injection comprise actively phagocytic cells (macrophages, mesothelial cells) wherein a high concentration of virus is achieved. It is likely that t does perform, *in vivo*, an essential function in SV40 transformation, and this function is probably related to its putative ability, *in vitro*, to induce the resting cell to traverse from G1 to S. The requirement for t may lie in its known association with pp2A phosphatase; the t-pp2A complex may affect the phosphorylated state of T antigen, and hence its ability to induce cell DNA replication.

B 307 EXPRESSION OF A SOLUBLE FORM OF *c-kit* IN TRANSGENIC MICE.

John McNeish, Don Cook and Oliver Smithies.
Department of Pathology, University of North Carolina, Chapel Hill, N.C. 27599.

The mouse W (white spotting) mutations are semi-dominant and cause developmental defects in early stages of the hematopoietic, pigment-forming and germinal cell lineages. W codes for a transmembrane receptor, *c-kit* (Chabot et al., 1988, Geissler et al., 1988). As a step toward understanding the interaction between the *c-kit* receptor and its ligand, we have constructed a plasmid encoding a soluble form of the receptor which lacks the transmembrane and cytoplasmic domains. CHO cells expressing this truncated gene secrete a glycosylated form of the *c-kit* receptor with correct molecular weight into the culture supernatant. To determine whether this soluble *c-kit* protein can interact with its endogenous ligand *in vivo* and thereby mimic the phenotypic effects observed in W animals, we are producing transgenic mice that express the soluble *c-kit* gene. The phenotype of these transgenic mice will be discussed.

Chabot et al., (1988) *Nature*, **335**, 88-89.
Geissler et al., (1988) *Cell*, **55**, 185-192.

B 308 ABBREVIATED SCRAPIE INCUBATION TIMES IN MICE EXPRESSING *Prn-p*^{b} TRANSGENES, Carol Mirenda, David Westaway, Dallas Foster, Michael Scott, George Carlson and Stanley B. Prusiner,

Departments of Neurology and of Biochemistry and Biophysics, University of California, San Francico, CA 94143 and McLaughlin Research Institute, Great Falls, MT 59401

The *Prn-i* gene controlling scrapie incubation times in mice is linked to the *Prn-p* gene which encodes the prion protein (PrP). Prototypic long (I/LnJ: >200 d) and short (NZW: ca. 120 d) incubation period mice have PrP genes, *Prn-p*^{b} and *Prn-p*^{a} respectively, differing at codons 108 and 189. Expression of hamster (Ha) PrP transgenes renders mice susceptible to Ha prion inocula; transgenic (Tg) HaPrP mice have incubation times and pathology typical of hamsters. These Tg-HaPrP mice have prolonged incubation times with mouse prion inocula. This suggests that *Prn-p* and *Prn-i* may be congruent. To test this hypothesis, we have created Tg mice in which *Prn-p*^{b} transgenes are superimposed on a short scrapie incubation time background. Tg-*Prn-p*^{b} mice have scrapie incubation times dramatically shorter (85 ± 6.2 d, n = 16) than non-Tg control mice (129 ± 5.2 d, n = 13), in contrast to ca. 200 d incubation periods predicted by classical genetic analysis. This unexpected result is unlikely to reflect chromosomal "position effects" on transgene expression, as three Tg lines, which are assumed to have distinct Tg insertion sites, behave in a similar manner.

B 309 *IN VIVO* ACTIVATION OF HIV-1 LONG TERMINAL REPEAT BY UV-B, PSORALEN-DEPENDENT UV-A IRRADIATION AND VARIOUS SKIN APPLICANTS IN TRANSGENIC MICE, John D. Morrey*, Samuel M. Bourn*, Thomas D. Bunch*, Robert W. Sidwell*, Louis R. Barrows¶, Raymond A. Daynes# and Craig A. Rosen§. *AIDS Research Program, Utah State University, Logan, Utah 84322-5600, ¶Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84132, #Department of Pathology, University of Utah and §Department of Molecular Oncology and Virology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Ultraviolet-B (UV-B) irradiation activates the human immunodeficiency virus-1 (HIV-1) long terminal repeat (LTR) in cell culture. To evaluate the effect of UV-B, and also UV-A irradiation *in vivo*, transgenic mice were made to contain the HIV-1 LTR linked to firefly luciferase reporter gene. Both UV-B (333 J m^{-2}) and psoralen-dependent UV-A (30,000 J m^{-2}) irradiation activated the HIV LTR/luciferase gene 2-3 $\log_{10}$ above background levels. Luciferase reporter gene activity was found to be activated in the skin of transgenic mice even after a short exposed to sunlight. Moreover, the LTR was activated in the skin of transgenic mice administered an oral dosage of psoralen and exposure to UV-A irradiation. Unexpectedly, some chemicals, such as dimethyl sulfoxide (DMSO), and some commonly used pharmacological skin application substances activate the LTR without exposure to UV light. These findings suggest that UV exposure, the clinical usage of photosensitizing pharmacologic substances and some skin application substances in HIV-infected people might be contraindicative because of the possibility of enhancing HIV infection. (Supported by NIH NIAID Contract NO1-AI-72662)

B 310 TRANSGENIC MICE EXPRESSING SENSE AND ANTISENSE-MYB CONSTRUCTS, E.W. Newcomb[1], *F. Grignani[2], G. Inghirami[2], K. Feng-Zeng[1] and R. Dalla-Favera[2], [1]Department of Pathology and Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016; [2]Department of Pathology , Columbia University, New York, NY 10032.

Expression of c-myb is involved in the differentiation of hematopoietic cells and alterations in c-myb have been associated with hematopoietic malignancies. To define more precisely the role of c-myb in the B-cell lineage and to investigate the effect of deregulated expression of c-myb on B-cell differentiation, we constructed a series of transgenic mice expressing the following human c-myb sequences under the control of B-cell tissue specific enhancer and promoter elements: 1) normal c-myb coding domain constitutively expressed (MYB-D); 2) a truncated 3' version of c-myb coding domain analogous to v-myb, the oncogene of the avian myeloblastosis virus (MYB-T); and 3) an antisense construct spanning the entire c-myb coding region (MYB-AS). Eleven transgenic mouse lines were generated by the injection of the human c-myb gene constructs into C57BL/6J X DBA/2 fertilized embryos. Eight of the eleven lines examined to date (1/3 MYB-D, 1/2 MYB-T and 4/6 MYB-AS) express the transgene as assessed by RNAse protection assays. No tumors have been observed in any of the transgenic lines for a period of one year.Lymphoid tissues (spleen, bone marrow and mesenteric lymph node) from each of the transgenic lines will be analyzed for several T (LyT1 and Thy1.2) and B (B220, anti-IgM,IgG,and IgD) cell surface markers to determine whether pertubations in B-cell maturation occur in these mice.

B 311 EVOLUTIONARY CONCEPTS IN ONCOLOGY, Shinichi Okuyama and Hitoshi Mishina; Department of Radiology, Tohoku Rosai Hospital, Sendai 981, Japan

Cancer is now a disease of the gene. The human genome may contain genetic information representing its history of evolution. The gists of our presentation are as follows: (1) Carcinogenesis in man takes place as if it echoes the history of evolution: following the atomic bomb detonation in Hiroshima and Nagasaki in 1945, leukemias first appeared, cancers of the lung and stomach then, and breast cancer and choriocarcinoma, cancers of the mammalian symbol organs, the last (Cancer incidence in the population of Nagasaki City 30 years after atomic bombing, Tohoku J. Exp. Med., 155, 23-39, 1988). (2) Clinical aspects of malignancies are those of phylogenetic retrogression (devolution). Radiosensitivity of cancer arises as it simulates the animal life in the primitive sea: increased cellular water content as we can see it on MRI, decreased SOD (Probable superoxide therapy of experimental cancer with D-penicillamine, ditto, 135, 213-216, 1981), and defective DNA repair (Evolutionary concept of cancer in radiotherapy. Kokenshi, 37, 125-131, 1985). These evolutionary concepts would probably help us deciphering multioncogene mechanism of carcinogenesis, planning strategies against carcinogenic accidents as the Chernobyl, and developing more effective anticancer means (Evolution of Cancer, University of Tokyo Press, Tokyo, 1990).

B 312 BCR/ABL EXPRESSION IN TRANSGENIC MICE, Jan Willem Voncken, Nora Heisterkamp, Guido Jenster, Dimitris Kioussis, Paul Pattengale and John Groffen, Department of Pathology Research and Laboratory Medicine, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

The translocation which leads to the formation of the Philadelphia chromosome, an acquired chromosomal abnormality widely implicated in human leukemia, juxtaposes part of the ABL proto-oncogene (9q34) in a head-to-tail fashion to the 5' exons of the BCR gene (22q11). Depending on the type of leukemia (CML or ALL), the amount of BCR exons contained within the resulting novel chimeric gene appears to vary. To study these oncogenes in a more direct way, BCR/ABL hybrid genes, encoding the P210 or P190 protein, were constructed to generate transgenic mice. Transgene expression directed by the BCR gene promotor leads to embryo mortality presumably directly related to as well the expression of the BCR gene in the course of embryogenesis as to as for yet unkown pleiotropic lethal effects of the fusion gene during this period of development. Expression controlled by metallothionein promoter sequences, reveals evidence for neoplastic malignancies attributed to tumorigenic properties of the fusion gene.

Gene Regulation, Gene Therapy, Biotechnology

B 400 A TRANSGENIC MODEL SYSTEM TO STUDY PERIPHERAL NERVE REGENERATION.

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Our lab has adopted a molecular approach to examine the cells that participate in the repair of damaged nerves of the peripheral nervous system. At least three cell types have been implicated in the repair process of the mammalian sciatic nerve. These include myelinating and nonmyelinating Schwann cells as well as nonresident macrophages. To examine the role these cells play in nerve repair, we wish to employ the technique of cell ablation, in which transcriptional control regions that are active in the cells of interest are used to direct the expression of toxin molecules in transgenic mice. We have selected the control regions of the apolipoprotein (ApoE) gene, the nerve growth factor receptor (NGFR) gene, and the peripheral myelin protein P0 gene. The thymidine kinase gene of the Herpes simplex virus (HSV-TK), whose toxic effect is induced by the nucleoside analogue acyclovir, has been used as the toxin gene in these studies. Here we report the characterization of several transgenic mouse lines that express TK from these promoters. These lines will be helpful in studying the affect the induced ablation of macrophages or either type of Schwann cell has on nerve regeneration.

B 401 EXPRESSION OF BOVINE α-LACTALBUMIN IN THE MILK OF TRANSGENIC MICE CONTAINING A 6.4 KILOBASE GENOMIC CLONE OF BOVINE α-LACTALBUMIN.

Gregory T. Bleck and Robert D. Bremel. Endocrinology-Reproductive Physiology Program and Department of Dairy Science. University of Wisconsin-Madison.

The milk protein α-lactalbumin is essential for the biosynthesis of lactose in the mammary gland. Transgenic mice were produced to study the regulation of bovine α-lactalbumin gene expression. The gene encoding the milk protein bovine α-lactalbumin was isolated from a bovine genomic library. A 8.0 kilobase fragment containing the α-lactalbumin gene was purified and microinjected. The 8.0 kilobase fragment contains 2.1 kilobases of 5' flanking region, the 1.7 kilobase coding region and 2.6 kilobases of 3' flanking region. Three of fifty-one live offspring were identified as being transgenic using polymerase chain reaction. Second generation animals from one line were milked and analysis was performed using an enzyme linked immunosorbent assay for bovine α-lactalbumin. Bovine α-lactalbumin was present at a concentration of 0.5 μg protein/ml mouse milk. The animals produced will allow us to study the promoter strength as well as regulation of the gene in a transgenic model.

B 402 *Abstract Withdrawn*

B 403 DEVELOPMENTAL CHANGES IN THE METHYLATION PATTERN OF REGULATORY ELEMENTS OF THE MURINE ALPHA 1 TYPE I COLLAGEN GENE, [1]Michael Breindl, [1]Katherine Rhodes, [2]Richard A. Rippe and [2]David A. Brenner, [1]Department of Biology, San Diego State University, and [2]Department of Medicine, University of California, San Diego, CA 92182.

We have analyzed the methylation status of regulatory elements in the 5'-flanking region and the first intron of the murine alpha 1 type I collagen (COL1A1) gene by Southern blot hybridization experiments and a polymerase chain reaction assay. Sequences located >1 kb upstream of the COL1A1 promoter were methylated, and sequences in the first intron were unmethylated, in all cells and tissues analyzed, and the methylation status of these regions appeared not to be correlated with transcriptional activity of the COL1A1 gene. Only in a short region (< 0.8 kb) surrounding the start site of transcription, including the COL1A1 promoter and first exon, developmental changes in the methylation pattern were observed. This region was unmethylated in sperm DNA and became transiently de novo methylated in undifferentiated early embryonic cells. In adult tissues regulatory sites in the COL1A1 promoter were demethylated in cells derived from all three germ layers, independent of transcriptional activity of the gene. In contrast, a site located downstream of the promoter in the first exon was unmethylated in collagen-producing cells, but methylated in nonproducing cells, and methylation of this site thus appeared to be decisive for transcriptional activity of the promoter. DNase protection and gel shift assays indicated that DNA methylation does not affect COL1A1 gene transcription by directly interfering with interactions between transcription factors and regulatory DNA sequences. Rather,in cells not expressing the COL1A1 gene methylation may induce changes in the chromatin structure that make the COL1A1 promoter inaccessible to trans-acting factors and result in transcriptional suppression of the gene.

B 404 **EXPRESSION OF THE HUMAN PULMONARY SURFACTANT GENE SP-C IN TRANSGENIC MICE.** **Stephan W. Glasser, Thomas R. Korfhagen, Susan E. Wert, Michael D. Bruno and Jeffrey A. Whitsett. Division of Pulmonary Biology, Children's Hospital Medical Center, Cincinnati, Ohio 45229.**

SP-C is a hydrophobic peptide that is an important component of the pulmonary surfactant. SP-C is synthesized by Type II epithelial cells lining the alveolar epithelium. Transgenic mice were utilized to demonstrate the lung-specific expression of bacterial chloramphenicol acetyltransferase (CAT) under control of 5'-segments of the human pulmonary surfactant protein C gene. In constructs containing 3.7 kb of 5' sequence CAT activity was first detected on day 11 of gestation in fetal mouse lung. CAT expression increased dramatically reaching adult levels at the time of birth. Transgenic mice containing deletion constructs of this human SP-C genomic fragment demonstrated that 0.6 kb of DNA proximal to the TATAA box conferred lung-specific CAT expression but at levels several orders of magnitude less than the 3.7 kb fragment. The 0.6 SP-C-CAT did not support CAT expression in mouse lung prior to birth. Organ culture of fetal lungs (day 12 and 13 of gestation) from the transgenic mice were utilized to demonstrate enhanced expression of the 3.7 SP-C-CAT transgene during organ culture from 0-4 days. Dexamethasone increased SP-C-CAT expression in the organ cultures 3-4 fold. Discrete cis-active elements specify gene expression in the distal respiratory tract and confer high-level and tissue-specific expression of the chimeric genes in epithelial cells of the developing lung. Supported by Program of Excellence in Molecular Biology HL41496 and the Cystic Fibrosis Foundation.

B 405 HSV-MEDIATED GENE TRANSFER INTO RAT CNS, William F. Goins[1], Larry R. Sternberg[1], Marina Mata[2], David J. Fink[2] and Joseph C. Glorioso[1], Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261[1] and Department of Neurology, VAMC, University of Michigan, Ann Arbor, MI 48104[2].

Herpes simplex virus (HSV) displays a natural propensity to establish life-long latent infections within neurons, characterized by the exclusive production of the latency associated transcripts (LATs) and the complete absence of lytic viral gene expression. We have exploited these attributes to engineer recombinant HSV of reduced neuropathogenicity carrying recombinant gene cassettes to serve as vectors for the transfer and expression of foreign genes in the mature CNS. The E. coli β-galactosidase (β-gal) gene (lacZ) was used as a foreign gene, driven by either a viral late gene (gC) promoter or the latency specific (LAT) promoter to indicate productive and latent infection respectively. Recombinant HSV vectors were stereotactically injected into rat hippocampus. A mutant deficient in the US3 gene product, US3::pgC-lacZ, expressed β-gal in dentate gyrus neurons of the injected hippocampus from 2 to 5 days after injection. Unlike wild-type virus, this recombinant vector neither caused focal brain destruction nor spread throughout the brain. PCR and immunohistochemical analyses have shown that this viral mutant can be maintained in the latent state within hippocampal neurons as late as 10 months post injection without neuronal cell damage. Two other viral mutants, tk$^-$::pLAT-lacZ and d120::pLAT-lacZ (ICP4$^-$), have been used as vectors in focal brain injections. These two HSV mutants carry mutations in gene products that are required for replication of the virus following reactivation from latently infected neurons. In addition, the ICP4 gene product is essential for virus replication in vitro. These two viral vectors do not cause focal brain pathology and express β-gal long-term at times consistent with viral latency. These results demonstrate that apathogenic HSV vectors can be used to express foreign genes in cells of the brain, and we are now in the process of introducing foreign genes into these vectors that may prove useful in genetic therapy approaches to ameliorate inborn errors of metabolism that result in disorders of the CNS.

B 406 **TRANSGENIC RABBITS CARRYING MOUSE MAMMARY TUMOUR VIRUS LTR-HUMAN GROWTH HORMONE FUSION GENE.** Charanjeet Guron, S.K.Jain, Maneesh Taneja, Gurpreet Singh, S.M. Totey and G.P. Talwar. National Institute of Immunology, New Delhi 110 067, India

Mouse mammary tumour virus-long terminal repeat (MMTV-LTR)-human growth hormone (hGH) fusion gene has been constructed with an objective to assess the suitability of the gene construct to make a regulatable mammary gland specific transgenic expression system. The clone pLTGH10 contains a MMTV-LTR promoter (1.5kb) 5′ to the hGH gene (2.0kb). MMTV-LTR-hGH fusion gene was taken out as a 3.8kb HindIII-Eco R1 fragment later used for pronuclear injection into the rabbit zygotes to establish the founder transgenics.

Rabbit genomic DNA prepared from the blood of the putative transgenics and the normal controls was digested with HindIII and was analysed on the Southern blots hybridized with radiolabelled hGH gene fragment (2.0kb). The autoradiography signal shows the presence of two bands of very high molecular weight which are present in all the samples analysed including the normal controls. The presence of the autoradiography signal of more than 3.8kb legth in HindIII digested genomic DNA of putative transgenic rabbit pups suggests that a female rabbit pup is carrying the injected gene construct which is probably integrated into the genomic DNA of the transgenic rabbit.

B 407 THE USE OF TRANSGENIC MICE CONTAINING LACI/LAMBDA SHUTTLE VECTORS TO STUDY IN VIVO EFFECTS OF MUTAGEN EXPOSURE, Steven W. Kohler, G. Scott Provost, Annabeth Fieck, Patricia L. Kretz, and Jay M. Short, Stratagene Cloning Systems, 11099 N. Torrey Pines Rd. La Jolla, CA. 92037.

Transgenic mice have been generated which contain a lambda shuttle vector to study the effects of mutagen exposure in vivo. The shuttle vector contains the $lacI^q$ gene which serves as the target for mutagenesis. Following treatment of the animals, the target genes are recovered from genomic DNA prepared from a variety of tissues of the mice using in vitro lambda phage packaging extract. Mutations in the target gene are detected by infecting E. coli lawns on plates containing Xgal. Plaques with mutations in the $lacI^q$ gene appear blue while plaques with intact $lacI^q$ genes are colorless. The ratio of blue/colorless plaques is used as a measure of the mutagenicity of the compound. This system has been used to assess the mutagenicity of several compounds including N-ethyl-N-nitrosourea, cyclophosphamide, and benza(o)pyrine. A large induction of mutant plaques over background levels was detected for each compound. The shuttle vector in the mice also contains in vivo excision functions of the lambda ZAP™ vector which allow for rapid excision of the target gene from phage to plasmid. We have used this to determine specific nucleic acid alterations responsible for the mutant phenotype. It is anticipated that data from this system will add significantly to the existing pool of mutagenesis data as well as be valuable in providing insight into the origins of spontaneous mutations in vivo.

B 408 IDENTIFICATION OF A PHOTORECEPTOR/PINEALOCYTE-SPECIFIC PROMOTER THROUGH TRANSGENIC MICE. G. I. Liou, M. R. Al-Ubaidi, S. Matragoon, G. Hanten, W. Baehr, T. Yokoyama, and P. A. Over-beek. Cullen Eye Institute and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

A retina/pineal-specific promoter for the human gene encoding interphotoreceptor retinoid-binding protein (IRBP) has been identified through the use of transgenic mice: Analysis of six transgenic families inheriting a transgene composed of the human IRBP promoter fused to the CAT gene revealed that the CAT gene, concomitant with the endogenous IRBP gene, was expressed only in the retina and pineal gland. Three transgenic mouse families harboring human IRBP promoter-c-myc oncogene were generated. The morphological study of the developing retina (p20) in one of the families revealed a degenerative pattern in the outer nuclear layer. At p100, photoreceptors, outer nuclear and outer plexi-form layers are degenerated. All other retinal cells remain mophologically similar to non-transgenic littermate. Studies on the regulation of genes encoding photoreceptor-specific proteins such as IRBP should provide insights into the retinal development at the molecular level.

B 409 EXPRESSION OF BIOMEDICAL PROTEINS IN MILK OF TRANSGENIC ANIMALS, Platenburg G.J., Krimpenfort P., Kooiman P., Kootwijk H., Nuyens J., Pieper F., Warmerdam G., Woloshuk S., de Boer H.A., Strijker R., GenePharming Europe B.V. and Dept. of Biochemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

Production of biomedical proteins in milk of transgenic dairy animals may have advantages over classical production methods. The mammary glands of these animals are extremely efficient in secreting proteins and downstream processing of milk is relatively simple. In addition it has been shown that mammary gland epithelial cells are capable of modifying proteins post-translationally. As a first step in establishing this technology we are determining specific DNA sequences capable of directing the expression of heterologous proteins to the mammary gland. We have cloned and partially sequenced large regions flanking the bovine αS1-casein gene. This is a milk-specific protein expressed at very high levels (10 mg/ml). Expression studies in mammalian cellines have shown that the sequences we have cloned contain all the elements necessary to drive expression of a heterologous reporter gene. Sequences between 680 nt upstream and 180 nt downstream of the transcription initiation site of the αS1-casein gene are sufficient for this expression although sequences more upstream are possibly involved in efficiency of expression in the mammary gland. We have shown that several proteins bind to the DNA region between -680 and +180 of this casein gene. Some sequences that are recognized by these proteins are present in all casein genes sequenced so far and may be involved in mammary gland-specific expression. As a candidate gene to be expressed in the mammary gland of transgenic animals we have cloned the human lactoferrin gene. This gene codes for a iron-binding protein with strong anti-bacterial activity. In humans the protein may play a role in iron resorption by the infant as well as in establishing the composition of the intestinal flora. Expression vectors that contain different portions of the αS1-casein regulatory sequences linked to the hLF cDNA have been injected into mouse zygotes and transgenic animals have been generated. RNA analysis shows that the casein/hLF constructs are tissue-specifically expressed. Milk samples of transgenic mice were analysed and contain high levels of hLF. Transgenic hLF behaves as a stable whey protein and is indistinguishable of lactoferrin purified from human milk according to different functional and immunological assays. Details of the analysis will be presented on the poster.

B 410 **RETROVIRAL-MEDIATED TRANSFER AND EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN DOG SKIN FIBROBLASTS,** N. Ramesh and William R.A. Osborne, Department of Pediatrics, University of Washington, Seattle, WA 98195.

The fatal immunodeficiency associated with the inherited absence of adenosine deaminase (ADA) is potentially treatable by engraftment of transduced autologous skin fibroblasts. The capacity of skin fibroblasts to function as vehicles for gene transfer was investigated using two retroviral vectors, LASNL and LAENL, both of which have hADA controlled by viral LTR and contain a selectable Neo gene flanked by SV40 promoter and 5' untranslated region of Encephalomyocarditis virus sequences respectively. Genetically modified dog skin fibroblasts were cast in collagen matrices and were implanted subcutaneously or used as dermal equivalents in wound covering in the back of dogs. Removal of skin tissue from the graft showed hADA gene expression up to 3-fold higher than the endogenous level during the first two weeks which gradually reduced to 5-20% of the endogenous levels by week 8. Simultaneously the NPT expression was reduced as evidenced by decreased NPT activity and loss of G418 antibiotic resistance in fibroblast cultures reinitiated from the skin grafts. The suppression was not reversed by in vitro culture of the fibroblasts. Such supression was not observed in long term culture of transduced dog skin fibroblasts in vitro. hADA was found in tissue adjacent to the collagen matriaces, showing that migration of transduced fibroblasts away from the graft site may account for some of the reduction in hADA activity. Newer vectors with different control elements to overcome this problem are being designed and tested to achieve persistant expression of transduced genes in skin fibroblasts after transplantation.

B 411 MURINE SHORT-CHAIN ACYL-COENZYME A DEHYDROGENASE (SCADH) DEFICIENCY: ACTIVITY AND ANTIGEN ARE ABSENT IN MULTIPLE TISSUES FROM THE BALB/cByJ MOUSE, W.J. Rhead, B.A. Amendt, C.A. Reece and P.A. Wood, Dept. of Pediatrics, Univ. of Iowa, Iowa City, IA 52242 and Dept. of Comparative Medicine, Univ. of Alabama, Birmingham, AL 35294

SCADH deficient BALB/cByJ mice have a distinctive organic aciduria similar to that seen in human SCADH deficiency. Acyl-CoA dehydrogenase (ADH) activities were assayed in liver, muscle, and fibroblast mitochondria from homozygous control (Y) and SCADH deficient (J) mice. Long-chain ADH and medium-chain ADH (MCADH) activities were comparable in both groups in all tissues. In the presence of monospecific MCADH antiserum, SCADH activity was absent in all J mouse tissues. In J mouse fibroblasts, [9,10(n)-^{3}H]- and [15,16(n)-^{3}H]palmitate oxidations were 93% and 55% of control, respectively, also consistent with an isolated SCADH deficiency. We performed immunoblotting in liver, muscle and fibroblast mitochondrial matrix using monospecific antisera to MCADH and SCADH. We detected comparable amounts of MCADH antigen in all Y and J mice tissues. However, in J mice, SCADH antigen was undetectable in all three tissues. Since SCADH is deficient in multiple tissues derived from J mice, isolated muscular SCADH deficiency would not be expected in human SCADH deficiency. In support of this view, SCADH activity and [15,16(n)-^{3}H]palmitate oxidation were normal in fibroblasts from an adult female with apparent muscular SCADH deficiency (Turnbull et al, NEJM 311:1231, 1984). We are presently exploring the molecular defects in both murine and human SCADH deficiency.

B 412 NONSENSE MUTATION SUPPRESSION OF INTRODUCED AMBER NONSENSE MUTATIONS IN THE DIPHTHERIA TOXIN A-CHAIN GENE. Donald F. Robinson and Ian H. Maxwell.
Program in Molecular Biology, University of Colorado Health Sciences Center, Denver, CO 80262

Cell to cell communication plays an important role in the development of specific cell and tissue types. Ablation of specific cell types is a useful method of studying the effects on the development of the surrounding tissues and also for determining the ontogeny of cell lineages. We have previously shown that specific tissues can be ablated through tissue specific targeting of expression of the diphtheria toxin A-chain gene (DT-A). Here we present data on the use of nonsense mutation suppression to control the expression of functional DT-A. Through the suppression of an amber (TAG) stop codon introduced into the DT-A coding sequence, expression of a cotransfected firefly luciferase reporter gene is inhibited to 30 % of control levels in which no suppressor tRNA is supplied. Equivalent amounts of transfected wild type DT-A DNA, or of a double nonsense mutant, inhibit luciferase expression to 3% and 60% of control, respectively. By combining activation dependent expression with nonsense mutation suppression in a binary system of control, we hope to overcome the detrimental effects of low level ectopic DT-A expression from tissue specific promoter/enhancer elements.

B 413 PREVENTION OF ATHEROSCLEROSIS IN TRANSGENIC MICE WHICH OVER-EXPRESS HUMAN APOLIPOPROTEIN AI, E. Rubin, R. M. Krauss, J. Verstuyft, S. M. Clift, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Apolipoprotein AI (AI), the primary protein component of high density lipoprotein (HDL), is suspected to have a major effect on determining the level of HDL in plasma, the structure of the HDL particle, and on the susceptibility of an individual to develop atherosclerosis. In this study, we examined the effect of high AI expression by introducing the human AI gene into the atherosclerosis susceptible inbred mouse strain C57BL/6 (B6). In two independent transgenic lines, plasma levels of total AI and HDL are increased to twice that of non-transgenic littermates. The level of endogenous mouse AI in plasma from the transgenic animals is markedly reduced, by up to 10 fold, and contributes only 4% to the total plasma AI mass. There is no significant reduction in mouse AI mRNA compared to non-transgenic animals, indicating that the alteration of murine AI in the transgenic mice is regulated post-transcriptionally. The transgenic mice exhibit two distinct HDL populations, identical in size to the human size subclasses HDL_{2b} and HDL_{3a}, instead of the distinct single-sized HDL population present in control B6 mice. The B6 strain exhibit a reduced HDL level when on a high fat diet, an analogous situation to that seen in humans at increased risk for developing atherosclerotic heart disease. A1 transgenic mice were placed on a high fat diet for 18 weeks; the mice did not develop the atherosclerotic plaques found in the non-transgenic B6 controls on the same diet. Our results in mice transgenic for human A1 indicate 1) the importance of AI in determining the level and particle size of HDL, 2) the existence of a post transcriptional mechanism which alters the plasma level of endogenous mouse AI, and 3) high expression of human AI protects B6 mice from developing atherosclerosis.

B 414 THE SKELETAL α-ACTIN PROMOTER MEDIATES A DEVELOPMENTAL TRANSITION OF ACTIN EXPRESSION WHILE CYTOPLASMIC β-ACTIN PROMOTER PRODUCES EARLY EMBRYONIC AND GERM CELL EXPRESSION IN TRANSGENIC MICE, Arthur T. Sands, Franco De Mayo, Xin Lei, and Robert J. Schwartz, Department of Cell Biology, Baylor College of Medicine, Houston, TX, 77030

The tissue restricted and developmental potentiation of transcription by chicken α-skeletal and β-cytoplasmic actin promoter regions fused to chloramphenicol acetyl transferase and lacZ reporter genes were characterized in transgenic mice. The patterns of transgene expression were compared to the pattern of gene expression of the endogenous mouse α-skeletal, α-cardiac, and β-cytoplamic actins as revealed by Northern blotting of total RNA from embryonic, fetal, and adult mice. Seven of ten transgenic mice containing the chicken α-skeletal actin promoter fused to a reporter gene (CAT or lacZ) resulted in preferential transgene transcription in skeletal muscle tissue, similar to the endogenous mouse α-skeletal actin gene. Three of ten transgenic lines departed from the preferred pattern of skeletal muscle expression with primary expression of the α-skeletal actin-reporter transgene in tissues containing primarily cardiac or smooth muscle actin isoforms: the heart or the vasculature. In transgenic CAT lines, a developmental transition from embryonic heart to fetal and neonatal skeletal muscle expression was produced by the transgene promoter, a pattern of developmental regulation similar to that of the endogenous α-skeletal actin gene. β-Actin-lacZ transgenic lines produced a pattern of preferential transgene expression in the germ cells and the pre-implantation mouse embryo. In five of ten transgenic lines, chicken β-actin lacZ expression in pre-implantation embryos first appeared at the two-cell stage of embryogenesis and was reduced at the morula and blastula stages as detected by X-GAL staining. In these lines, significant staining was not apparent at later embryonic stages. In the adult, expression was detected in the germ cells of the testis of seven lines and the brain of three lines. Alternately, one line demonstrated high transgene expression in skeletal muscle and heart with no concomitant expression in the testis or brain. Patterns of β-actin-lacZ transgene expression displayed significant similarities to the endogenous mouse β-actin gene.

B 415 DEVELOPMENTAL AND FUNCTIONAL ANALYSIS OF THE MOUSE HOMOLOGUE OF THE TUMOR-ASSOCIATED MUCIN PEM, Andrew P. Spicer and Sandra J. Gendler, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

High molecular weight mucin glycoproteins are expressed by a wide variety of epithelial tissues and are often important differentiation markers in the development of these tissues. The polymorphic epithelial mucin, PEM, is a highly glycosylated apical membrane glycoprotein expressed by simple secretory epithelial tissues, e.g. lactating mammary gland, pancreas, lung, fallopian tube, salivary gland and chief cells of the stomach. It is also highly expressed by carcinomas where it shows aberrant processing (Burchell et al. (1989) Int. J. Ca. 44, 691). Although mucins have been assumed to have protective and/or lubrication roles in secretory epithelial tissues, the fact that nearly all adenocarcinomas express high levels of this particular mucin may suggest additional functions. Thus, in an attempt to elucidate the function, the mouse homologue has been cloned with a view to studying its role during development, differentiation and tumorigenesis. Interestingly, the mouse gene has undergone substantial evolutionary changes, although the potential for glycosylation remains high. The human core protein is mainly composed of precisely maintained 60 bp tandem repeats which code for a potentially highly glycosylated 20 amino acid repeat unit (Gendler et al. (1990) JBC 265, 15286). The repeat domain of the mouse gene differs from that of the human in codon and amino acid sequence, the length and number of some repeats and the location of the repeats within the protein. Thus, the homology between human and mouse repeats is <50% at the protein level. Regions of highest homology (87%) include the transmembrane and cytoplasmic domains. This homology is not surprising as the cytoplasmic tail interacts either directly or indirectly with the actin cytoskeleton (Perry et al. (1990) Exp. Cell Res.188,302), suggesting functional conservation of the sequence. Experiments are in progress to disrupt the function of the protein using homologous recombination in embryonic stem cells.

B 416 CORTICOTROPIN-RELEASING FACTOR (CRF) TRANSGENICS: A MOUSE MODEL OF CHRONIC CRF AND GLUCOCORTICOID OVERPRODUCTION WHICH MIMICS CUSHING'S SYNDROME, Mary Stenzel-Poore and Wylie Vale, Clayton Foundation for Peptide Biology, The Salk Institute for Biological Studies, La Jolla, CA 92037.

CRF, a hypothalamic peptide which is released in response to a variety of stimuli including stress, is a potent stimulator of pituitary adrenocorticotropin (ACTH) secretion and thus involved in the regulation of circulating glucocorticoid levels. The expression and release of CRF is ultimately negatively controlled by ACTH and glucocorticoids. Perturbations in this regulatory loop which result in excess quantities of glucocorticoids lead to Cushing's syndrome. To generate an animal model of chronic overexpression of CRF and glucocorticoids we have made transgenic mice containing a CRF transgene comprised of the metallothionein promoter /CRF structural gene. Of the 22 founders, three females exhibit abnormalities such as lethargy, obesity and bilateral, symmetric hair loss. These animals show significantly elevated serum glucocorticoids compared to nonstressed normal controls (20-30 fold) and have not bred successfully. One male also shows hair loss and moderate obesity but has produced F1 progeny. Animals which overproduce CRF will be used to investigate the effect of CRF on corticotroph development and the endocrine and nonendocrine roles of CRF. In addition, the effects of long term elevations of ACTH and glucocorticoids on the physiology of stress will be examined. These animals may provide a useful model of Cushing's syndrome.

B 417 TRANSCRIPTIONAL ACTIVATION BY SITE-DIRECTED PHOSPHORYLATION MUTANTS OF THE HUMAN PROGESTERONE RECEPTOR, Diane Tasset, Glenn Takimoto and Kathryn B. Horwitz, Department of Medicine/Endocrinology, University of Colorado Health Sciences Center, Denver, CO 80262

The steroid hormone receptors are transcriptional enhancer factors that bind DNA in the presence of hormone by recognition of specific enhancer elements, or hormone response elements. The mechanism for activation of transcription by steroid hormone receptors involves protein interactions between the receptor and other cellular transcription factors. It is not known how the receptors interact with these protein factors to activate transcription, but it is possible that phosphorylation of the receptor may play a role. Phosphorylation has been shown to critically alter the function of other transcription factors such as CREB, Oct2, GAL4, MyoD and the serum response factor SRF. All steroid receptors, as well as other members of the superfamily, studied to date are phosphoproteins.

In cultured breast cancer cells and transfected cells the human progesterone receptor (hPR) is phosphorylated in a hormone-dependent manner. This phosphorylation has been mapped to serine residues in the N-terminal portion of the receptor by phosphoamino acid analysis and reverse-phase HPLC of tryptic peptides. In these studies, we are using site-directed mutagenesis of specific serine residues to identify phosphorylation mutants. We have analyzed how phosphorylation mutant receptors affect activation of transcription on complex promoters where the PR interaction with other transcription factors is important, or on a minimal promoter where activation is due solely to the receptor.

Refs: Sheridan P.L. *et al* (1989) *J Biol Chem* 264:7054-7058 and references therein
Tasset D. *et al* (1990) *Cell* 62(6) and references therein

B 418 INDUCIBLE TISSUE SPECIFIC GENE EXPRESSION IN TRANSGENIC SYSTEMS.

Manfred Theisen (1), Fabienne Mischler (1), Dalila Ali-Hadji (1), Jean-Francois Spetz (1), Pierre Chambon (2) and Andrea Pavirani (1), (1) Transgene, 11, rue de Molsheim, 67000 Strasbourg, France and (2) Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasboug, France.

Transgenic animals can be used as model systems to study genetic disorders or pathological conditions that simulate human diseases. However, the expression of potentially "harmful" transgenes in transgenic organisms could be problematic. It may drastically influence the viability and fertility of animals and by that the possibility to establish and maintain the desired transgenic lines. Uncontrolled expression of lethal transgenes during embryonal development could lead to a loss of transgenic embryos prior to birth. The expression of the transgene under the control of inducible transcriptional elements could therefore be of advantage.

In a first attempt, to establish a model system for inducible tissue specific gene expression we have studied the steroid hormone dependent regulation of target transgene transcription in transgenic mouse liver by steroid hormone receptor derived trans-activators. Two independent groups of transgenic mice were generated. One contains the human α1-antitrypsin gene as target gene under the transcriptional control of a truncated β globin promoter that contains the yeast UAS G transcriptional enhancer.The other contains the gene for the hybrid trans-activator GAL/ER that consists of the DNA binding domain of the yeast trans-activator Gal 4 and the hormone binding and activating domains of the human estrogen receptor (Webster et al., 1988, Cell 54, 199 - 207) under the control of the liver specific mouse transferrin promoter. This fusion receptor activates transcription from a UAS G containing promoter in an estrogen dependent manner. Double transgenic animals were generated by breeding, that contain both the GAL/ER receptor transgene and the a1-antitrypsin target gene.

Data for the estrogen dependent regulation of α1-antitrypsin expression by the GAL/ER fusion receptor in livers of these mice will be presented. Further applications of the inducible system and the use of additional trans-activators will be discussed.

B 419 PAROTID SPECIFIC EXPRESSION OF THE HUMAN *AMY1C* GENE IN TRANSGENIC MICE,

C.-N. Ting, L. C. Samuelson, M. P. Rosenberg*, and M. H. Meisler, Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109-0618 and *Dept. Molec. Biology, Bristol-Meyers Squibb Pharmaceutical Research Institute,PO Box 4000, Princeton, NJ 08543

Human amylase is encoded by a multigene family which includes 3 salivary genes (*AMY1A*, *AMY1B*, and *AMY1C*) and 2 pancreatic genes (*AMY2A* and *AMY2B*). The proximal 5'-flanking regions of these genes contain two inserted elements. A γ-actin pseudogene is located 200 bp upstream of the first coding exon of all five genes. In addition, each salivary amylase gene contains a full-length endogenous retrovirus which inserted into the γ-actin pseudogene. Transcription of the *AMY1* genes is initiated within the γ-actin sequences, suggesting that the retrovirus activated a cryptic promoter for expression in the salivary gland. The amylase gene family was generated during primate evolution from one ancestral gene (MCB 10:2513). We are interested in the role of the inserted elements in the evolution of tissue-specific expression of these genes. Transgenic mice are being used to map the cis-acting DNA sequences necessary for expression of *AMY1C* in the parotid salivary gland. Tissue-specific expression in the parotid gland was observed after transfer of a cosmid clone containing the *AMY1C* structural gene, with 10 kb upstream sequences (including the retrovirus), and 11 kb of downstream sequences. To further localize the parotid-specifying element(s), a series of fusion constructs with the human growth hormone reporter gene has been transferred to transgenic lines. Preliminary results have localized the parotid-specifying element(s) to1.6 kb which includes retroviral sequences.

B 420 EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN RHESUS MONKEYS UPON AUTOLOGOUS TRANSPLANTATION OF BONE MARROW CELLS INFECTED WITH RECOMBINANT RETROVIRUSES,

Victor W. van Beusechem, Henny H. Lemmink and DinkoValerio, Inst. of Applied Radiobiology and Immunology-TNO, P.O. Box 5815, 2280 HV, Rijswijk, The Netherlands.

Congenital deficiency of adenosine deaminase (ADA) is considered one of the prime candidates for somatic cell gene therapy. For the development of such gene therapy protocols we have generated the recombinant retroviral vector LgAL(ΔMo+PyF101), in which sequences encoding human ADA are transcribed from a hybrid LTR in which the enhancer sequences from Mo-MuLV were replaced by an enhancer from the F101 host range mutant of polyoma virus. This vector exhibited promising expression patterns in the murine hemopoietic system following transplantation of infected pluripotent hemopoietic stem cells (Van Beusechem et al., J.Exp.Med. 172 (90):729). In order to extend these studies into a model more relevant for the clinical situation we performed autologous transplantations in rhesus monkeys using bone marrow that was infected with LgAL(ΔMo+PyF101) virus by co-cultivation with virus-producing fibroblasts for 90 hours during which the cells were stimulated with recombinant hemopoietic growth factors (human IL-1α and/or rhesus monkey IL-3). Successful human ADA gene transfer was first shown *in vitro*, by demonstrating ADA-overexpression in myeloid progenitor cells and by zymogram analysis on mature myeloid cell types cultured from the infected bone marrow. Upon autologous transplantation of the co-cultured bone marrow into the lethally irradiated monkeys we monitored the hemopoietic regeneration, which allowed the semiquantitative assessment of the repopulating capacity of the graft (Gerritsen et al., Transplantation 45 (88):470). Under optimal conditions the co-cultivation procedure did not significantly affect the regenerating capacity of the rhesus monkey bone marrow. All six monkeys transplanted so far expressed the functional human ADA enzyme in their peripheral blood cells, as detected by zymogram analysis. The levels as well as the duration (up to 12 weeks post transplantation) of expression varied depending on the hemopoietic growth factor(s) used during infection of the bone marrow. To further define the persistence of human ADA expression, polyclonal antisera specifically directed against human ADA were raised in rhesus monkeys. Long-term *in vivo* expression studies using these antisera are currently in progress.

B 421 MUTANT KERATIN EXPRESSION IN TRANSGENIC MICE CAUSES MARKED ABNORMALITIES RESEMBLING A HUMAN GENETIC SKIN DISEASE. Robert Vassar, Pierre A. Coulombe, Linda Degenstein, Kathryn Albers and Elaine Fuchs. Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637

As surface and lining cells, stratified squamous epithelial (SQE) cells share in common a protective function, manifested by the production of an extensive cytoskeletal network of 10 nm intermediate filaments (IF) composed of keratin. In epidermis, keratins can consitute up to 85% of the total protein of a terminally differentiated cell, and hence it has been of longstanding interest to know whether mutations in keratin genes might cause some of the myriad of human genetic skin diseases of unknown etiology. Using in vitro transfection of genes encoding truncated human keratins, we previously showed that even small amounts of mutant keratins are sufficient to cause dominant disruptions of endogenous keratin filament networks in cultured epidermal cells. We have now expressed both truncated and wild-type human keratin proteins specifically in the SQE tissues of transgenic mice. To track the expression of human keratins, we replaced C-terminal sequences with those encoding the antigenic portion of neuropeptide substance P. Using immunoblot analysis, we quantitated the relative levels of mutant and wild-type keratins in transgenic epidermis. Using immunohistochemistry and immunoelectron microscopy, we examined the SQE tissues of the affected mice. To fully understand our in vivo results, we have (a) conducted in vitro filament assembly studies on mixtures of wild-type and truncated mutant keratins; and (b) cultured keratinocytes from these transgenic mice. Mice expressing the truncated, but not the wild-type, keratin exhibited gross abnormalities in tissue architecture and keratin filament formation within SQE such as epidermis and tongue. The morphology, pathology and biochemistry of the transgenic mice and their cultured keratinocytes bore a striking resemblance to a class of genetic skin diseases.

B 422 CYTOKERATIN GENE EXPRESSION IN TRANSGENIC MICE

Miguel A. Vidal, Angel Ramírez, Ana Bravo, Federico Baeza and José L. Jorcano
Department of Molecular and Cellular Biology, CIEMAT, Madrid

Cytokeratins define a family of 20-30 polypeptides that constitutes the cytoskeleton of intermediate filaments characteristic of epithelial cells. Based in structural andfunctional criteria, these polypeptides are divided into two subfamilies: acidic or type I and basic or type II cytokeratins. Filaments are heteroplymers formed from an equal number of subunits from each of these two subfamilies. Specific pairs of certain basic and acidic polypeptides have been identified in the various types of epithelial cells and the different epithelia are characterized by the combination of the cytokeratin pairs that are synthesized.
To investigate the regulatory elements for this differential, cell-type specific gene expression we have introduced bovine cytokeratin genes into the germ line of mice. Genes encoding for keratins present in stratified epithelia were used to construct gene fusions with E. coli lacZ as well as chimeric cytokeratin genes. Lines carrying the different transgenes have been generated and work is in progress to analyze their expression patterns.

B 423 CHONDRODYSPLASIAS IN TRANSGENIC MICE DUE TO MUTATIONS IN THE TYPE II COLLAGEN GENE, E. Vuorio, S. Garofalo, M. Metsäranta, L. Garrett, J. Vaughan, G. Lozano, D. Toman, M. Machado, W. Horton and B. de Crombrugghe, Department of Molecular Genetics, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Type II collagen is the major protein in hyaline cartilage. The supramolecular form of this homotrimeric protein is a fibril providing structural strength to cartilage. We have isolated full length cosmid clones for the mouse type II collagen gene, sequenced the entire gene (>31 kb), and mutagenized specific regions of the gene coding for functionally important domains of the protein. These include Gly residue at position 85 of the triple helix, two Lys residues which participate in the covalent intra- and intermolecular cross-links, and exon 7 coding for 15 amino acids near the N-terminal end of the triple helix. Transgenic mice harboring these mutations have exhibited variable phenotypes resembling human chondrodysplasias. Characteristic features include short limbs, hypoplastic cartilages, micrognathia, cleft palate and abnormally short snout. In light microscopy the epiphyseal growth plates appeared disorganized. Electron microscopy revealed abnormal fibrils and extended endoplasmic reticulum in chondrocytes. PCR amplification of cDNAs made from total RNA showed that the mutant transgenes are expressed in cartilaginous tissues.

B 424 THE PHARMOKINETICS OF B-GALACTOSIDE INDUCERS IN MAMMALIAN SYSTEMS, Denise L. Wyborski, Jay M. Short, Stratagene Cloning Systems, La Jolla, CA 92037

In E.coli the lac repressor provides strong repression of lac transcription by binding to the operator sequence positioned immediately downstream of the lac promoter. Induction of the repressed operon is achieved by binding the repressor molecule to certain B-galactosides. Studies have shown that the lac repressor system is functional in mammalian cells, and that binding of the repressor to operator DNA can be relieved by IPTG, a known inducer. Interest in modifying the lac repressor system for use in transgenic animals prompted examination of the pharmokinetics of inducer molecules in whole animals and in cultured cells. Time for maximal uptake of inducer by cultured cells was determined, followed by measurements of the clearance rate of the inducers from the cells. Cytosolic versus nuclear uptake by the cells was also measured. Uptake of B-galactosides by various mouse tissues was calculated, saturation kinetics of the tissue cells with inducer was measured, and the rate of inducer clearance from the blood was determined. The effect of the mouse metabolism on the structure and function of the B-galactosides was examined by HPLC and an E.coli activity assay. This increased understanding of B-galactoside inducer activity will aid in the development of a modified lac repressor system to control gene expression in transgenic animals.

B 425 IN VIVO AND IN VITRO GENE TRANSFER TO MAMMALIAN SOMATIC CELLS BY PARTICLEBOMBARDMENT, Ning-Sun Yang, Joseph K. Burkholder, Elizabeth B. Roberts, Brian J. Martinell and Dennis E. McCabe, Agracetus, Inc., Middleton, WI 53562

Chimeric chloramphenicol acetyltransferase (CAT), β-galactosidase (β-Gal) and luciferase (Lux) marker genes were coated onto fine gold particles and electrostatically bombarded into mammalian tissues and cells. Transient expression of the genes was obtained in liver, skin and muscle tissues of rat and mouse bombarded in vivo. Similar results were obtained with freshly isolated rat and human mammary gland ductal segments and their derived primary cultures. Gene transfer and transient expression was also observed in 8 different human cell culture lines that originated from epithelial, endothelial, fibroblast or lymphocyte cells. Using CHO and MCF-7 cell cultures as models, we obtained stable gene transfer at frequencies of $1.7x10^{-3}$ and $6x10^{-4}$, respectively. The particle bombardment technology thus provides a useful and versatile means to transfer foreign genes into a variety of mammalian somatic cell systems. The method is applicable to tissues in situ as well as to isolated cells in culture and has proven effective with all cell or tissue types tested thus far. This technology may therefore prove to be applicable to various aspects of gene therapy.

Late Abstracts

PERINATAL LETHALITY (PLE): A MUTATION CAUSED BY INTEGRATION OF A TRANSGENE INTO DISTAL MOUSE CHROMOSOME 15.

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We have identified a transgenic mouse line in which a recessive insertional mutation has occurred as a result of transgene integration into a locus required for post-natal viability. Newborn mice homozygous for the transgene are smaller than their sibs, feed poorly, and generally die within one day after birth. Pathological analysis of these mice reveals that their livers have variable, but often quite severe, vacuolization of hepatocytes. The histochemical and ultrastructural appearance of the affected livers is not suggestive of known carbohydrate or lipid storage disorders, or of a respiratory disorder. The cellular damage appears similar to that seen in toxic liver injury.

We have used recombination analysis to map the position of the transgene in the mouse genome. It is very tightly linked to a cluster of loci that include the mutations caracul (Ca) and microcytic anemia (mk). This region is also linked to the int-1 proto-oncogene, the Hox-3 gene family, and the cytokeratin type II gene family; this synteny is conserved with the homologous genes found on human chromosome 12.

We call the mutated locus in the transgenic line ple, for perinatal lethality. We have cloned the genomic sequences which flank the transgene insertion site and are analyzing this region in order to identify the disrupted gene. This should facilitate the characterization of the molecular basis of the presumptive metabolic defect. The conservation of synteny further suggests that there may be a homolog for this locus on human chromosome 12.

HTBF1 IS A BASIC PROTEIN BINDING TO THE HUMAN TELOMER SEQUENCE (TTAGGG)n, Claudio Brigati, Dina Balderes and David Shore,

Dept of Microbiology, College of Physicians and Surgeons of Columbia University 701 W.168th Street, New York, N.Y. 10032 USA;*Present Address: Laboratorio di Biologia Molecolare, I.S.T., Viale Benedetto XV, n°10, 16132 GENOVA

By screening a HeLa cDNA expression lybrary with a double stranded trypanosome telomer, we have found a partial cDNA clone encoding a basic protein binding to the repeat TTAGGG. The binding is shown by the ability of protein extracts from bacterial lysogens harboring the phage or the "in vitro" translated product to specifically complex with a double stranded multimerized TTAGGG probe in a gel shift assay.

We are now expressing the protein in bacteria and Baculoviral systems in order to produce polyclonal antibodies that will possibly label the ends of human metaphase chromosomes. We discuss the possible roles of this protein in gene expression or in influencing chromosomal structure.

IDENTIFICATION AND CLONING OF TTF-1, A T LYMPHOCYTE-SPECIFIC TRANSCRIPTION FACTOR CONTAINING A SEQUENCE-SPECIFIC HMG BOX. **Hans Clevers, Mariette Oosterwegel, Dennis Dooijes, and Marc van de Wetering. Department of Clinical Immunology, University Hospital Utrecht, p.o.box 85500, 3508 GA, Utrecht, The Netherlands.**

CD3-ε gene expression is controlled by a downstream T lymphocyte-specific enhancer element. We report the identification of a T cell-specific transcription factor, TTF-1, binding to this element. The multimerized recognition motif of TTF-1 constituted a T cell-specific enhancer. Subsequent cloning of TTF-1 identified three splice alternatives. TTF-1 contained a single DNA-binding HMG box most closely related to similar boxes in the putative mammalian sex-determining gene SRY and in the S. pombe Mc Mating Type gene. TTF-1 mRNA was expressed uniquely in T lymphocytes. Upon cotransfection into non-T cells, TTF-1 could transactivate transcription through its cognate motif. These results identify TTF-1 as a T cell-specific transcription factor, which might play a role in the establishment of the mature T cell phenotype.

A MUTATION IN THE C-MYC PROTO-ONCOGENE INTRODUCED BY GENE TARGETING IS LETHAL IN HOMOZYGOUS EMBRYOS. A.Davis and A.Bradley. Institute for Molecular Genetics. Baylor College of Medicine. Houston, TX. 77030.

The c-myc proto-oncogene protein product has been characterized as a phosphorylated nuclear protein which is expressed throughout the cell cycle. The gene is differentially expressed in the embryo and the expression pattern has been characterized from day 5.5 to day 14.5 of gestation. Despite a large body of work, the function of this protein is still unknown. We have used a replacement vector with positive, negative selection to target a mutation into the c-myc gene in ES cells. Germ-line chimeras from two separate targeted clones were bred and the heterozygous offspring were phenotypically normal. On crossing of heterozygotes, no homozygous offspring were born in 135 pups tested. The c-myc mutation, which introduces a neo gene 48 amino acids after the AUG translation initiation codon, appears to be lethal in the homozygous state at approximately 9.5 days of gestation.

EXTREME LYMPH NODE HYPERPLASIA EXHIBITED IN A TRANSGENIC MOUSE LINE EXPRESSING IL-7.

Barry L. Davison, Philip J. Morrissey, Carol Ware, Cynthia R. Willis, Blair Renshaw and Jacques Peschon, Departments of Transgenics and Immunology, Immunex Corporation, Seattle, WA 98101.

A transgenic mouse line, #1201, has been established in which lymphocyte-specific interleukin-7 (IL-7) expression is mediated by the murine *lck* promoter element. In this lineage IL-7 expression is predominantly directed to the thymocyte compartment resulting in altered lymphoid phenotype. Extensive Peyer's patch hyperplasia has been detected at three weeks postpartum while more generalized lymph node hyperplasia is detectable at around 3 months. These animals die from complications associated with severely enlarged mesenteric lymph nodes which attain weights of 6-8 grams. Surface antigen analysis of such hyperplasias indicates that the majority of the cells (> 99%) represent a non α/β TCR bearing, early thymocyte class. Analysis of genomic DNA from these cells indicates extensive γ/δ gene rearrangement. These cells are tumorigenic when transplanted into nude mice resulting in extreme lymph node hyperplasia.

TARGETED GENE DISRUPTION OF THE MURINE CD45 GENE IN EMBRYONIC STEM CELLS,

Eileen A. Elliott and Richard A. Flavell,
Department of Immunobiology, HHMI, Yale University School of Medicine, New Haven, CT 06510

The leukocyte common antigen (L-CA, CD45) is composed of a family of tyrosine-specific phosphatases which are abundantly expressed by cells of hematopoietic origin. CD45 is a large cell surface glycoprotein encoded by a single locus found on chromosome 1 in both mouse and humans. Numerous isoforms of CD45 have been identified and shown to have derived from the alternate splicing of three exons which encode sequences near the amino terminal portion of the molecule. The function of CD45 is currently unknown but has been proposed to play a role in the activation of T and B lymphocytes.

In our approach to test this hypothesis we are attempting to generate mice deficient in the expression of CD45, or which are limited in the isoforms of CD45 expressed, by the use of homologous recombination in mouse embryonic stem cells. Toward this end we have generated a number of PNS-replacement-type targeting vectors containing neomycin cassettes disrupting exons 2, 6 or 9. These constructs have been introduced into embryonic stem cells and putitive homologous recombination events are being further characterized.

TARGETED DNA INSERTION INTO MAMMALIAN GENOMES BY THE CRE-LOX SITE SPECIFIC RECOMBINATION OF PHAGE P1. Shinichi Fukushige & Brian Sauer, Molecular Biology, CR&D Dept., E. I. du Pont de Nemours & Co., P. O. Box 80328, Wilmington, DE 19880-0328

Direct introduction of DNA into mammalian cells has proven to be a powerful method for the analysis of gene function and regulation. However, position effects and variation of copy number which accompanies DNA integration events obtained using traditional gene transfer methods prevent precise analysis. To circumvent these problems, we have introduced the Cre-*lox* site specific recombination system into mammalian cells.

Cre is a 38kDa recombinase from bacteriophage P1 that catalyzes reciprocal site specific recombination between 34 bp *loxP* sites. The Cre protein can mediate intramolecular excision and inversion events as well as intermolecular (integrative) recombination. Our previous results indicated that Cre can perform not only efficient excisive intramolecular site specific recombination but also integration of an exogenous circular DNA at a chromosomal *loxP* site in both yeast and mammalian cells. We have used a derivative of the murine Ltk$^-$ cell line containing an SV40 promoter 5' to a *loxP* site to demonstrate the integration of a promoterless *lox* tk integrating vector. Transient expression of the *cre* gene induces the site specific integration of the *lox* tk plasmid into the chromosomal *loxP* site to reconstruct a functional *tk* gene. Significantly, the resulting site directed integrants were predominantly simple single copy insertions. These results suggest that Cre-mediated site specific integration will allow us to analyze the function of a DNA sequence reproducibly by eliminating variation from such position and copy number effects.

Here we describe a general integrating vector in which a functional *neo*^{r} gene is reconstructed after Cre-*lox* site specific recombination. This vector will be useful in a variety of cell lines after placement of the appropriate target *lox* site into the genome.

EXPRESSION OF POLYOMAVIRUS MIDDLE T ANTIGEN IN THE MAMMARY GLANDS OF TRANSGENIC MICE RESULTS IN THE INDUCTION OF POLYCLONAL MAMMARY ADENOCARCINOMAS. Chantale Guy and William J. Muller, The Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Transgenic mice carrying a cDNA encoding the polyomavirus middle T antigen linked to the Mouse Mammary tumor virus promoter/enhancer (MMTV) were derived to assess the consequences of ectopic expression of middle T antigen and its associated tyrosine kinase in the mammary epithelium. Of the eleven independent transgenic founder animals, four have developed mammary carcinomas that involve the entire epithelium. In the best characterized line TG.MT-5, mammary gland specific expression of middle T antigen resulted in the rapid appearance of tumors (4-5 weeks of age) involving all mammary glands in every transgene carrier thus far analysed. Histological analysis of these tumors and surrounding tissues revealed the complete absence of any morphologically normal mammary epithelium. The polyclonal appearence of these tumours suggests that expression of middle T antigen alone is sufficient to transform the mammary epithelium.

INTESTINAL HYPERPLASIA IN TRANSGENIC MICE CONTAINING FATTY ACID BINDING PROTEIN/SV40 T-ANTIGEN FUSION GENES. S.M. Hauft, K.A. Roth, S. Harris, S. Rees, S. Cohn, J.R. Hansbrough, G.H. Schmidt and J.I. Gordon, Depts. of Pediatrics, Biochemistry and Molecular Biophysics, Washington University, St. Louis, MO 63110, and Genetics Dept., Glaxo, Greenford, Middlesex, England UB6 OHE

The intestinal epithelium is composed a monolayer of cells undergoing rapid and continuous proliferation and differentiation. Stem cells located in the crypts of Lieberkuhn give rise to daughter cells that differentiate to form the 4 mature cell types of the gut epithelium. Differentiation occurs as the cells migrate away from the zone of proliferation in the crypts. Migration from crypt to villus tip takes 2-3 days in the adult mouse intestine. Cells are extruded at the villus tip. An interesting question of gut epithelial biology is how this balance between proliferation and differentiation is maintained. Our previous studies have utilized portions of the 5' nontranscribed domains of the rat intestinal (I-) and liver fatty acid binding protein (L-FABP) genes to direct appropriate cell- and region-specific expression of a reporter, human growth hormone, in transgenic mice. We have now constructed fusion genes using our I-FABP (-1128 to +28) and L-FABP (-596 to +21) promoter sequences with the SV40 T-antigen (T-ag) reporter to examine the effects of the T-ag on the balance of proliferation and differentiation in the gut epithelium. Several pedigrees of transgenic mice containing each fusion gene have been analyzed, demonstrating the expression of T-ag is limited to the intestine and liver (for L-FABP/T-ag). Expression of the T-ag in the intestine results in an increase in weight and length. Histologic studies show an increase in epithelial cellularity and an expansion of the zone of proliferation outside of the crypt to the lower villus. In L-FABP/T-ag transgenic mice hepatocytes show marked nuclear atypia and pleomorphism: these changes were not observed in the intestinal epithelial cells. Expression of the intact endogenous I- and L-FABP genes are not affected by the presence of the transgene.

INTRACEREBRAL GRAFTING OF GENETICALLY MODIFIED FIBROBLASTS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE. H.A. Jinnah, L.J. Fisher, T. Friedmann, and F.H. Gage. Departments of Neurosciences and Pediatrics, University of California San Diego, La Jolla, CA 92093.

We have been developing methods for the intracerebral grafting of genetically modified cells to correct the neurological abnormalities in Parkinson's disease. In the current study, primary fibroblasts were obtained from a skin biopsy from inbred Fischer rats and infected with a retroviral vector containing the neomycin-resistance gene as a selectable marker and the cDNA for rat tyrosine hydroxylase (TH), the rate-limiting enzyme in the production of dopamine. Cells were selected in G418, and a subclone (FF2/TH) which could be immunostained with an antibody to TH and which expressed functional TH activity in vitro was isolated. The ability of these cells to produce a phenotypic effect on behavior was then examined in an animal model of Parkinson's disease. Rats were given unilateral lesions of the dopaminergic nigrostriatal pathway with the neurotoxin 6-hydroxydopamine, and abnormal apomorphine-induced rotational behavior was quantified. FF2/TH cells or control cells were then implanted stereotactically into the rostral caudoputamen. Two to eight weeks after grafting, the rotational responses of animals with control grafts remained unchanged. In contrast, rats with FF2/TH grafts showed a 75% decrease in abnormal rotational behavior 2 weeks after grafting, with a gradual rise to 45% decrease by 8 weeks. Histological examination at 10 weeks after grafting revealed good survival of FF2/TH cells. In addition, in situ hybridization and immunohistochemical studies demonstrated that the cells continued to produce TH mRNA and TH protein. These results suggest that fibroblasts infected with a cDNA for TH can continue to express TH for at least 10 weeks and can produce partial phenotypic correction of behavioral abnormalities in an animal model of Parkinson's disease.

DOMINANT POSITIVE AND NEGATIVE SELECTION USING A HYGROMYCIN PHOSPHOTRANSFERASE-THYMIDINE KINASE FUSION GENE.

Stephen D. Lupton, Laurie L. Brunton, Victoria A. Kalberg, and Robert W. Overell. Department of Molecular Biology, Immunex Corporation, 51 University Street, Seattle, WA 98101-2977. A selectable gene that functions effectively in a dominant manner for both positive and negative selection in mammalian cells has not been reported previously. To create such a gene, the bacterial hygromycin phosphotransferase (*hph*) gene was fused in-frame with the *Herpes simplex* virus type I thymidine kinase (HSV-I TK) gene. The fusion gene (termed HyTK) encodes a bifunctional protein that confers hygromycin B resistance (Hm^r) and ganciclovir sensitivity (GCV^s), and provides a means whereby dominant positive and negative selectable phenotypes may be expressed and regulated as a single genetic entity. Plasmid vectors containing the HyTK gene were constructed and used to demonstrate the efficacy of the HyTK gene for positive and negative selection in NIH/3T3 and Rat-2 cells. The HyTK gene was slightly more effective than the *hph* gene at conferring Hm^r in both NIH/3T3 and Rat-2 cells. When compared with the HSV-I TK gene in Rat-2 cells, the HyTK gene was slightly less effective at conferring the ability to grow in HAT medium (HAT^r), but markedly more effective at conferring GCV^s. Retroviral vectors were constructed containing the HyTK gene. High titer virus stocks were generated, which conferred both Hm^r and HAT^r on infected cells. Infected cells contained unrearranged proviruses and were killed (>99%) by GCV. The HyTK gene should be of significant value as a 'suicide gene' in gene therapy applications because the HyTK gene permits graft ablation with GCV. The HyTK gene may also be used to perform 'in-out' homologous recombination in wild-type cells.

EXPRESSION OF A NOVEL HOMEOBOX GENE IN HEAD FOLD NEUROECTODERM OF THE EARLY MOUSE EMBRYO,

Kathleen A. Mahon[1], Edit Hermesz[1], and Milan Jamrich[2], [1]Laboratory of Mammalian Genes and Development, NICHD, NIH, and [2]Division of Biochemistry and Biophysics, CBER, FDA, Bethesda, MD 20892

Embryonic patterning along the antero-posterior axis in *Drosophila* is controlled in part by complexes of homeobox containing genes. The expression pattern of the known murine homeobox genes suggests that a similar gene network may control development in this organism as well, particularly in defining regional (and perhaps segmental) identity in the developing central nervous system (CNS). The *Antennapedia*-like homeobox (*Hox*) genes are expressed in specific domains of the CNS, each with a distinct rostral boundary in the neural tube or hindbrain. The mouse *engrailed*-like genes, *en*-1 and *en*-2, have expression boundaries extending more anteriorly into the mesencephalon. We have isolated a new gene from the mouse that contains a homeobox similar to those of the *Drosophila paired and gooseberry* genes. *In situ* hybridization experiments have shown that expression of the "prd-like" gene in 8.5 day embryos is restricted to the rostral neuroectoderm of the head fold. "Prd-like" is unique in that its domain of expression is the most anterior of all known homeobox containing genes. This striking pattern of expression suggests a role in the early patterning of the prosencephalon and may provide insight into the segmental nature of the developing CNS.

REGULATION OF CLASS I MHC GENE EXPRESSION IN CHORIOCARCINOMA CELLS,

John R. Mendiola and Harry T. Orr, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis MN 55455.

Choriocarcinoma cell lines are derived from extraembryonic fetal trophoblasts. During gestation, certain populations of trophoblasts contact maternal tissue. Like trophoblasts, the choriocarcinoma cell line JEG-3 does not express HLA-A and -B genes but does express the relatively nonpolymorphic HLA-G gene. HLA-G expression is largely restricted to trophoblasts. We have found that the 1.5 kb upstream fragment of HLA-G can direct transcription of the reporter gene CAT in JEG-3 cells, but not in B lymphoblastoid cells which do not express their endogenous HLA-G genes. These results suggest that the 1.5 kb HLA-G 5' fragment contains a positive transcriptional element(s) active in JEG-3 choriocarcinoma cells but not in B lymphoblastoid cells which may be at least partially responsible for specific expression of HLA-G in trophoblasts. Deletional studies are being pursued to more precisely define these elements. We have also found that a 550 bp fragment of DNA from the upstream region of the HLA-A2 gene which contains a defined H2TF1 binding enhancer is able to activate transcription of CAT in both JEG-3 choriocarcinoma cells and B lymphoblastoid cells. These data, along with transgenic mouse data, suggest that additional negative regulatory elements are present and functional in cytotrophoblasts which prevent expression of classical class I genes in these cells. We currently are testing fragments of DNA further upstream to identify elements that normally act to prevent HLA-A expression in JEG-3 cells which were not present on our shorter HLA-A2-CAT constructs. Characterization of HLA-G regulation may provide an opportunity to characterize novel developmentally regulated transcriptional control elements, while characterization of negative regulatory elements in HLA-A2 could give insight into disease states, such as certain virally induced cancers, which show a lack of expression of class I MHC products.

PRELIMINARY STUDIES TOWARDS TRANSGENIC ANALYSIS OF THE DNA REPAIR GENE *ERCC5*, John S. Mudgett, Joyce Nickols, Rudy Hernandez, and Mark A. MacInnes, Genetics Group, Los Alamos National Laboratory, Los Alamos, NM 87545

The complete 32 kbp human nucleotide excision repair gene *ERCC5*, of complementation group 5, was previously isolated as a functional gene on overlapping cosmids (Mudgett and MacInnes, Genomics vol. 8, 1990). The human *ERCC5* DNA repair gene complemented the genetic defects of UV-sensitive (*ERCC5*-deficient) hamster and mouse host cells, demonstrating functional conservation of this repair gene. Specific unique sequence regions of *ERCC5* were used to isolate human *ERCC5* cDNA clones, and these fragments were shown to be conserved cross-species to hamster and mouse genomic DNA by Southern analysis. The conserved human *ERCC5* fragments and the human *ERCC5* cDNA insert were used to screen mouse cDNA and genomic libraries for isolation of the mouse *ERCC5* DNA repair gene. Putative mouse *ERCC5* clones have been isolated and are being characterized. Homologous recombination vectors will be constructed to inactivate functional domains of the mouse repair gene in cultured mouse fibroblasts and embryonic stem cells. Resulting homozygous *ERCC5*-deficient mouse cell lines will be used to characterize the role(s) of various *ERCC5* domains in cell viability, repair, mutagenesis, and *in vitro* embryonic development. (Supported by the U.S. DOE under contract W-7405-ENG-36, and JSM by an Alexander Hollaender Postdoctoral Fellowship award).

DISRUPTION OF IL-3 GENE BY HOMOLOGOUS RECOMBINATION IN EMBRYONIC STEM CELLS AND GENERATION OF CHIMERIC MICE, Richard Murray, Choy-Pik Chiu, Terrill McClanahan, and Frank Lee, Department of Molecular Biology, DNAX Research Institute, Palo Alto, CA 94304-1104

The Interleukin (IL) 3 gene product was first identified as a soluble molecule capable of stimulating splenic lymphocytes. Numerous studies have subsequently revealed a broad spectrum of activities on cells of hemopoietic origin. Notably, IL-3 appears to be active on early progenitor cells of the bone marrow. The production of IL-3 in the hemopoietic system is primarily restricted to stimulated T cells, although IL-3 also appears to be expressed in brain, suggesting a possible neurological role. To clarify the in vivo functions of this molecule, we have inactivated one allele of the IL-3 gene in embryonic stem (ES) cells so that mice deficient in IL-3 production can be created. Presently, one ES clone has been isolated where IL-3 has been inactivated via homologous recombination. To accomplish this poly A traps and the "double selection" method were used, decreasing random recombination by 100 fold. One hundred fifty colonies surviving this selection were screened by Southern blot analysis, one of which was correctly targeted. These cells have been injected into C57Bl/6 blastocysts, and the first litters of chimeric mice have been produced. Breeding of these mice will reveal if the IL-3 targeted clone is capable of germline transmission.

JIMPY MUTANT MOUSE: TRANSGENIC EXPRESSION OF MYELIN PROTEOLIPID PROTEIN (PLP) IN A MOUSE MODEL OF PELIZAEUS-MERZBACHER DISEASE, Klaus-Armin Nave[1,3], Carol Readhead[2], Leroy Hood[2], and Greg Lemke[1], Molecular Neurobiology Lab, The Salk Institute, CA[1], California Institute of Technology[2], and ZMBH, University of Heidelberg, Fed. Rep. Germany[3].

The neurological mouse mutant *jimpy* (*jp*) is an accurate model for Pelizaeus-Merzbacher-Disease (PMD), an X-chromosome linked primary myelin-deficiency in humans. We have previously identified a point mutation in the *jp* gene for proteolipid protein (PLP), the major membrane protein of CNS myelin, which results in the aberrant splicing of *jp* PLP mRNA (PNAS 83:9465). Curiously, some of the reported ultrastructural abnormalities in the *jp* CNS precede the appearance of oligodendroglial cells (Skoff, Nature 264:560), and thus the expression of PLP during myelin formation. To determine whether the PLP gene is primarily affected in this mutation and in PMD, we have isolated the intact PLP gene, including 20 kb of 5' and 3' flanking sequences, from a mouse cosmid library, and generated several lines of PLP-transgenic mice. Male founder mice (PLP^{tg}) were bred with heterozygous (*jp*/+) female carriers to study the autosomal expression of this gene in genetically mutant mice. The phenotype and PLP expression of (PLP^{tg}/+, *jp*/Y) mice will be presented.

SEX-SPECIFIC MANIFESTATION OF PARENTAL IMPRINTING OF AN EXPRESSED *Hprt* TRANSGENE IN MICE, Dimitrina D. Pravtcheva*, Thomas L. Wise*, David W. Melton**, Frank H. Ruddle*, *Department of Biology, Yale University, New Haven CT 06511, and **Department of Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, United Kingdom

Some genes in the mouse are expressed differently depending on their maternal or paternal inheritance. The observation that transgenes may also show such differences in expression has provided an experimental model for the analysis of the molecular nature and biological significance of parental imprinting. We have found evidence for imprinting in a line of transgenic mice carrying a mouse Hprt (hypoxanthine guanine phosphoribosyl transferase) minigene at an autosomal or pseudoautosomal location. The injected clone represents the insert of the pDWM1 plasmid, and contains the promoter region, coding sequences and the last two introns of the mouse Hprt gene ($Hprt^b$ allele). Transgenic mice were bred to replace their X chromosomes with X chromosomes carrying an $Hprt^a$ allele, and transgene expression was determined by isoelectric focusing (IEF). Male progeny showed a single pattern of expression, irrespective of the route of transmission of the gene. In contrast, female progeny expressed the transgene differently when inherited through the mother and the father. The two patterns of expression in females differed in the apparent level of transgene activity and in the distribution of this activity between HPRT-B and HPRT-B/HPRT-A hybrid bands observed on IEF gels. We are currently investigating the role played by methylation in maintaining the different patterns of expression in transgenic female mice. We are also trying to determine (i) the time in embryonic development when the two expression patterns become manifest, and (ii) whether the absence of the Y or the presence of two vs. one X chromosome is responsible for the apparent sex specificity of the imprinting effect.

INTRODUCTION OF A SUBTLE MUTATION INTO THE MURINE *HOX-2.6* LOCUS BY THE HIT & RUN METHOD FOR GENE TARGETING IN ES CELLS. R.Ramírez-Solis and A. Bradley. Institute for Molecular Genetics. Baylor College of Medicine. Houston, TX. 77030.

Murine Hox genes are presumed to participate in embryonic development just as their homologous counterparts in *Drosophila* do; however formal proof does not exist since no mutant mice have been identified for these genes. Gene targeting by homologous recombination has allowed the production of mutations at the exact chromosomal location of several genes. Most of these mutations carry with themselves the presence of a selectable marker (often a *neo* gene) which creates a big mutation and frequently introduces potent regulatory elements that can alter the normal pattern of expression of the targeted and neighboring genes. This is not convenient for the study of the function of some genes, and the Hox gene family is a prototypic example of these. These genes exist linked in clusters, and the regulatory regions for each one are not completely known. The insertion of a marker gene can easily alter the the expression of other genes besides the targeted one and make a phenotype, if present, uninterpretable. We show that it is possible to introduce a small mutation into a non-selectable gene, *Hox-2.6*, and get ES cell clones that contain the desired mutation without the presence of any other alteration. These clones are able to generate chimaeric animals with a high contribution from the injected cells. The chimaeras are being mated to assess the potentiality for germ line transmission. The mutation consisted of the introduction of a 14 bp insertion that creates a stop codon in the middle of the putative recognition helix of the homeodomain of the *Hox-2.6* gene. This should disrupt the normal DNA binding capabilities of the protein and, hopefully, produce a visible phenotype. We will discuss the way the mutation was created and the chimera and germ line transmission data. R.R.S. acknowledges partial support from the C.I.B.M.y C.

HUMAN GROWTH HORMONE EXPRESSION IN TRANSGENIC MOUSE MILK, V.B. Reddy[1], J. Vitale[1], C.M. Wei[1], and J.M. Robl[2],[1] Transgenic Sciences, Inc., Worcester, MA 01608, [2] University of Massachusetts, Amherst, MA 01003

We have constructed a fusion gene containing mouse whey acidic protein (WAP) promoter and human growth hormone (hGH) coding sequences and microinjected into mouse embryos. A total of 24 transgenic mice were produced carrying multiple copies of WAP-hGH fusion genes. Of these six females and one male failed to produce FI offspring. Infertile transgenic females mated with males but did not carry litters to term. Of 17 remaining transgenics tested 11 gave birth to transgenic offspring. All but two of the transgenics expressed hGH in the serum ranging from 16ng/ml to 9500ng/ml. Four of the female transgenics produced hGH in their milk ranging from 65ng/ml to 0.41mg/ml. The high expressing line was propagated to generate homozygous offspring which expressed hGH in milk at levels as high as 3.5mg/ml. Although low levels of hGH were detected in serum during lactation in this line, growth and reproduction were normal. These results demonstrate the feasibility of producing commercially important levels of hGH in the milk of transgenic animals.

FUNCTIONAL ANALYSIS OF THE HUMAN ß-GLOBIN DOMINANT CONTROL REGION (DCR) HYPERSENSITIVE SITE 3. Dale J. Talbot, Sjaak Philipsen, Ali Amam, Peter Fraser and Frank Grosveld. National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, United Kingdom.

Hypersensitive site 3 is capable of conferring position independent, high level expression of a co-linked human ß-globin gene in Murine Erythroleukaemia cells and transgenic mice. Dissection of this site has separated two independent elements. The first is a strong enhancer element, characterized by two binding sites for AP1, which bind both nuclear factor erythroid 2 (NFE-2) and members of the AP1 family. This element, when cloned upstream of the human ß-globin gene, gives an intermediate enhancement of expression in MEL cells, but shows strong position effects in transgenic mice. The second element confers position independent expression, but at a low level in the absence of the enhancer element. This element is composed of three binding sites, one for nuclear factor erythroid 1 (NFE-1), and two for ubiquitous nuclear factors.

Talbot *et al.* (1990), EMBO J., vol 9, no. 7, pp. 2169 - 2178.

HIGH-LEVEL EXPRESSION OF BOVINE α S1 CASEIN cDNA UNDER THE CONTROL OF MMTV PROMOTER/ENHANCER IN THE MILK OF TRANSGENIC MICE.

Heng-Cherl Yom, Robert D. Bremel and Neal L. First+ Dept. of Dairy Science and Dept. of Meat and Animal Science+, University of Wisconsin, Madison, WI 53706

We have produced, by microinjection, 3 lines of transgenic mice carrying bovine α S1 casein cDNA under the control of mouse mammary tumor virus (MMTV) promoter/enhancer (5' flanking region), and SV40 early splicing region and polyadenylation site (3' flanking region). Transgenic mice were screened by Southern blot and polymerase chain reaction. Lactating offspring, from a male founder, expressed a high level of bovine α S1 casein (0.2 mg/ml) in their milk as determined by Western blot analysis using chicken anti-casein antibodies. These antibodies did not cross-react with any mouse milk proteins. The expression of the transgene in lactating mice was further induced by dexamethasone injection and analyzed by Western blot. The results showed that 0.5 - 0.6 mg/ ml of bovine casein was expressed into the mouse milk, indicating a 2.5 to 3 fold increase compared to that without induction. Northern blot analysis showed that the transgenes were transcribed at a similar level in the mammary glands of transgenic mice, when compared to the α S1 mRNA of lactating cow mammary glands.