TRANSGENIC MODELS IN MEDICINE AND AGRICULTURE

Organizer: Robert Church
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Analysis of Animal Development

F 001 GENETICS OF EARLY EMBRYO SURVIVAL, Lee Niswander[1], Della Yee[1], Eugene M. Rinchik[2] and Terry Magnuson[1], [1]Department of Genetics, Case Western Reserve University, Cleveland, OH 44106, and [2]Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831
The albino-deletion complex represents one of few regions of the mouse genome where a set of specific developmental defects are associated with a series of overlapping chromosomal deletions. A total of 37 deletions exist, all of which remove the region of mouse chromosome 7 that surrounds and includes the albino coat color locus. The complementation patterns among these deletion chromosomes indicate that at least 9 distinct units exist in this region. Four of these units are needed for normal embryonic development during the preimplantation or early postimplantation stages. Our work has concentrated on a morphological and molecular genetic analysis of two of these units, each of which is known to contain a gene(s) whose expression is required around the time that the basic body plan is being established in the early postimplantation embyro. Embryos homozygous for deletions that remove one of these units develop to day 8.5 where extensive development of the extraembryonic structures occurs, and the three primary germ layers form but there is no organization of mesoderm into somites or induction of the neural axis. Embryos homozygous for deletions that remove the second region do not undergo gastrulation and there is no development of the extraembryonic structures. Molecular markers for the region removed by the albino deletions have been generated by chromosome microdissection of a wild-type chromosome 7 followed by microcloning procedures. Preliminary analysis of 20 of the 68 microdissected clones containing single copy inserts indicates that 3, possibly 5, of the inserts map to the albino-deletion region. Experiments are underway to identify transcription units and pulsed-field analysis is being done to identify deletion breakpoints.

Gene Expression in Transgenics

F 002 SECRETION OF HETEROLOGOUS PEPTIDES IN TRANSGENIC ANIMALS. A.J. Clark, A.L. Archibald, H. Bessos*, P.Brown, S.Harris, M.McClenaghan, C.Prowse*, J.P.Simons, C.B.A. Whitelaw, I. Wilmut. AFRC Institute of Animal Physiology and Genetics Research, King's Buildings, West Mains Road, Edinburgh EH9 3JQ, Scotland. * Scottish National Blood Transfusion Service, Royal Infirmary, Edinburgh EH3 9HB, Scotland.
We are assessing the use of the mammary gland of transgenic animals for the production of large amounts of human proteins. Fusion genes have been constructed using sheep β-lactoglobulin to drive the expression of human Factor IX and human α1-antitrypsin. Transgenic mice are being used as a model system and transgenic sheep for potential large scale production. Results on the expression of Factor IX and a1-antitrypsin will be presented.

F 003 CANCER INITIATION IN THE LENS OF TRANSGENIC MICE, Heiner Westphal, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892. We have recently reported on lens tumors of transgenic mice that express SV40 oncogenes directed by a lens specific αA crystallin promoter (K.A. Mahon, A.N. Chepelinsky, J.S. Khillan, P.A. Overbeek, J. Piatigorsky, and H. Westphal, Science 235:1622-1628, 1987). My colleagues T. Nakamura, K. Mahon, T. Kuwabara and myself have now investigated the molecular genetics of cancer initiation in these mice. Transgenes consisting of αA crystallin promoter sequences fused to the transforming region of the SV40 genome were inserted in the mouse germ line. Two lines of transgenic mice, αT1 and αT2, differed sharply in cancer progression. All αT1 animals developed fast growing lens tumors and died young. By contrast, in αT2 mice, tumor progression was slow, and the animals lived a normal life span. Cancer progression correlated directly with the developmental state of the lens target tissue at the time of initial accumulation of oncogene products. In αT1, SV40 tumor antigens were detected at a very early stage of embryonic lens development, prior to lens fiber differentiation. By contrast, in αT2 mice, oncogene products were confined to newly differentiated fiber cells within the embryonic lens. We postulate that undifferentiated lens cells grow fast, hence the rapid cancer progression in αT1 mice. On the other hand, differentiated cells have a slow doubling time, hence the protracted tumor growth in αT2. SV40 transformed lens cells have been established in vitro from both αT1 and αT2 lenses. The αT2 lens cell lines continue to produce α, β, and γ crystallins, even after prolonged time in tissue culture. These cells have been found to contain factors that control crystallin gene activity.

Gene Transfer in Domestic Animals-I

F 004 EXPRESSION OF GROWTH-RELATED FUSION GENES IN PIGS. V.G. Pursel, K.F. Miller, D.J.Bolt, C.A. Pinkert,[1] R.E. Hammer,[1] K.E. Mayo,[2] R.D. Palmiter[3] and R.L. Brinster[1]. Agricultural Research Service, U.S.D.A., Beltsville, MD 20705; [1]University of Pennsylvania, Philadelphia, PA; [2]Northwestern University, Evanston, IL; and [3]University of Washington, Seattle, WA.

We have produced transgenic pigs that harbor structural genes for bovine (bGH) and human (hGH) growth hormone ligated to mouse metallothionein-1 (MT) promoter and human growth hormone releasing factor (hGRF) ligated to MT promoter or mouse albumin (ALB) promoter. Immunodetectable concentrations of foreign GH were present in blood plasma of more than 60% of the MT-hGH and MT-bGH transgenic pigs. Elevation of GRF was found in 2 of 8 MT-hGRF transgenic pigs and 3 of 3 ALB-hGRF transgenic pigs. Even though plasma concentrations of GRF in the GRF transgenic pigs were elevated 10-fold to 500-fold higher than in littermate controls, plasma concentrations of porcine GH (pGH) were not elevated in the GRF transgenic pigs. In contrast, plasma concentrations at birth ranged from 3 to 949 ng hGH/ml for MT-hGH transgenic pigs and 5 to 944 ng bGH/ml for MT-bGH transgenic pigs. Presence of the foreign GH depressed endogenous pGH to non-detectable levels. In MT-hGH and MT-bGH transgenic pigs, plasma concentrations of insulin-like growth factor-1 (IGF-1) were elevated more than two-fold above littermate control pigs, which indicates the foreign GH was able to bind to GH receptors in the liver to stimulate IGF-1 synthesis. Expression of the GH genes in pigs improved their efficiency of converting feed into body weight gain by 15% and resulted in marked repartitioning of nutrients from subcutaneous fat into other carcass components, including muscle, skin, bone and certain organs. The persistent excess GH in transgenic pigs was detrimental to general health; lameness, lethargy and gastric ulcers were the most prevalent problems. Transgenic females that expressed the hGH or bGH genes were anestrus. Several MT-bGH transgenic females ovulated in response to injections of exogenous gonadotropins. Germline transmission has been obtained from both expressing and non-expressing pigs. All transgenic progeny of MT-hGH, MT-bGH and MT-hGRF founder males expressed the transgene if their sire also expressed the gene.

F 005 DEVELOPMENT OF TRANSGENIC PIGS, Thomas E. Wagner, Edison Animal Biotechnology Center, Ohio University, Athens, Ohio 45701

Transgenic animals including mice, rabbits, sheep and pigs have been produced by direct microinjection of foreign DNA into the nuclei of single cell embryos (1). Because of the scientific value and potential economic importance of transgenic livestock, several attempts have been made to produce improved livestock using this technology. The principal structural gene transferred has been the growth hormone gene because increased serum levels of growth hormones have been shown to increase growth rates and the efficiency of feed utilization as well as to dramatically reduce body lipid in several livestock species (2). While transgenic mice expressing growth hormone are healthy, some livestock display severe infirmities when they contain an expressing growth hormone transgene (3). In order to regulate the expression of growth hormone to discrete periods during the life cycle of the pig an acutely regulatable P-enolpyruvate carboxykinase/bovine growth hormone gene (4) was introduced into the germ line of several lines of swine. The performance of these animals is compared to those expressing constitutively.

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2. Etherton, T.D., Wiggins, J.P., Chung, C.S., Evock, C.M., Rebhun, J.F. and Walton, P.E. J. Animal Science 63, 1389-1399 (1986).
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4. McGrane, M.M., deVente, J., Yun, J., Bloom, J., Park, E., Winshaw-Boris, A., Wagner, T., Rottman, F.M., and Hanson, R.W. The Journal of Biological Chemistry 263, 11443-11451 (1988).

F 006 THE PHYSIOLOGICAL CONSEQUENCES OF GROWTH HORMONE FUSION GENE EXPRESSION IN TRANSGENIC SHEEP, K.A. Ward, C.D. Nancarrow, J.D. Murray, P.C. Wynn, P. Speck and J.R.S. Hales, CSIRO, Division of Animal Production, PO Box 239, Blacktown, NSW, 2148, Australia.

An ovine metallothionein-Ia/ovine growth hormone fusion gene (MTSGH9) was introduced into 4 transgenic sheep by pronuclear microinjection[1]. Three females died at 2, 10 and 11 months of age. A surviving male is currently 6 months old. Here we report some physiological parameters, gene expression data and histological observations.

The plasma concentration of ovine growth hormone (GH) of the transgenic animals was 0.9 - 30 µg/ml, compared to control values of about 0.01 µg/ml, but their growth rates were always equal to or less than controls. The most striking effect[2] of the elevated GH levels was that total body fat content was only 1/2 to 1/5 of that in controls, due to basal metabolism (heat production) being elevated by 30%. The elevated heat production gave rise to a high core temperature (about 40°C). Cardiac output was elevated, but arterial blood flow was maintained at normal levels by a lowered total peripheral vascular resistance. Nitrogen balance was not different from controls, although the rate of protein turnover was elevated[3]. Elevated GH was associated with elevated insulin and Insulin-like Growth Factor-1, and a decrease in thyroid function. Testosterone and LH levels were normal when measured at 20 min. intervals over a 26 hr. period.

Tissues which contained ovine GH mRNA were, in decreasing order of significance: kidney, brain, liver (one animal only), lung, intestine, spleen and uterus. Gross abnormalities of the fore- and hind limbs were observed due to enlargement of joint capsules, eroded articular surfaces and a 45° rotation of the radius and ulna. Immediate cause of death was degenerative change in the kidney and liver. Much greater control over growth hormone expression is required before transgenic sheep containing growth hormone fusion genes will be viable or useful.

1. Nancarrow, C.D., Ward, K.A. and Murray, J.D. (1988). Aust. J. Biotechnol. 2, 39.
2. Graham, N.McC. and Margan, D.E. (1988). Proc. Nutr. Soc. Aust. 13, (in press).
3. Oddy, V.H., Warren, H.M. and Ewoldt, C.L. (1988). Proc. Nutr. Soc. Aust. 13, (in press).

Gene Transfer in Domestic Animals-II

F 007 EXPRESSION AND MOBILITY OF RETROVIRAL INSERTS IN THE CHICKEN GERM LINE, Lyman B. Crittenden, Regional Poultry Research Laboratory, 3606 East Mount Hope Road, East Lansing, Michigan, 48823; and Donald W. Salter Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824. We have introduced avian leukosis virus proviruses into the germ line of chickens by inoculating fertile eggs with recombinant viruses having RAV-0 LTRs, an endogenous virus, and the subgroup A envelope gene (1,2,3,4). Of the 23 initial inserts, two were defective for virus production. One produced non-infectious virions and a deletion was found in pol, but both gag and env genes were expressed. The other failed to produce gag products or virions but expressed env glycoproteins specific for subgroup A. Embryos and chickens carrying this insert were very resistant to subgroup A virus infection presumably due to interference. Thus, we have introduced a dominant gene for resistance to the common oncogenic subgroup of avian leukosis virus. Males and females carrying two of the inserts produced progeny carrying proviral inserts at other locations in the genome at high frequency, presumably through infection of germ cells (5). Thus, we have successfully introduced replication competent avian leukosis virus into the germ line by a relatively simple procedure.

1. Salter et al., 1986. Poultry Science 65:1445-1458.
2. Salter et al., 1987. Virology 157:236-240.
3. Hughes et al., 1986. Poultry Science 65:1459-1462.
4. Wright and Bennett, 1986. Virus Research 6:173-180.
5. Crittenden and Salter, 1988. Proceedings of the 2nd International Conference on Quantitative Genetics, pp. 207-214.

F 008 FACTORS INFLUENCING GENE EXPRESSION IN POULTRY, Ann M. Verrinder Gibbins and Cynthia L. Brazolot, Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. Factors are being studied which influence the expression in poultry of the major gene coding for the egg yolk protein precursor, vitellogenin (Vg). Vitellogenin is synthesized in the liver under the influence of estrogen, and is secreted into the blood. Subsequent transfer of Vg into oocytes is via receptor-mediated endocytosis; cleavage products of Vg are deposited in the egg as part of the yolk. A gene construct has been developed that incorporates sequences coding for a truncated version of Vg that can be detected immunologically. The construct includes all DNA elements known or suspected to be involved in the precise regulation of Vg gene expression. Accuracy of expression of the construct is being measured partly in vitro, but induction of the gene by estrogen and the secretion of newly synthesized protein is being tested in cell culture. Manipulation of various regulatory regions of the construct will allow more precise definition of their role in Vg gene expression. Of particular interest to us is the identity of the sequence(s) coding for the region(s) of Vg recognized by the oocyte membrane receptors. At present, these regions are poorly defined but, by manipulating certain regions of the construct and testing the resulting protein for uptake by oocytes, we hope to delineate the recognized regions. The corresponding DNA sequences and other Vg gene regulatory elements could be fused to genes coding for commercially valuable proteins; these products would be deposited in the egg, from which they could be harvested. Funded by NSERC, OEPMB, OMAF and NATO.

F 009 **EXPRESSION OF MUSCLE SPECIFIC GENES INTRODUCED INTO MOUSE GERM LINE.** **Moshe Shani, Institute of Animal Sciences, ARO, The Volcani Center, Bet-Dagan, 50-250, Israel.**

Terminal differentiation of muscle cells is characterized by the transition from proliferating mononucleated myoblasts to post-mitotic multinucleated myotubes. This morphological event is associated with the activation of a large battery of genes, whose products are involved in the formation of the sarcomere and in the provision of energy for muscle contraction. To define control mechanisms involved in the activation of muscle specific genes we are employing three gene transfer approaches, all of which can insert genes into the germ line: retroviral vectors, multipotent embryonic stem cell lines (ES) and transgenic mice.

Retrovitral vectors carrying the bacterial CAT gene driven by the internal rat skeletal muscle promoter were constructed, and high titers of recombinant viruses were produced. These viruses were used to infect cultures of the rat myogenic cell line L8. In most clones carriying the provirus, the expression of the CAT gene was stage-specific, regardless of the orientation of the internal promoter with respect to viral transcription. In addition, regulated expression was observed in clones selected and unselected for G418 resistance. These results indicate that internal promoters can function properly despite the close proximity of the viral control elements, and that the viral geneome is probably integrated into active chromosomal domains.

ES cells were established from normal mouse blastocysts. These cells can be induced to diffrentiate into most cell types both in vivo and in vitro. A Cloned muscle-specific gene introduced into these cells by electroporation was expressed specifically in myogenic cells derived from the transfected ES cells, provided that the DNA was introduced without vector DNA sequences. The presence of prokaryotic DNA sequences resulted in many cases in de novo methylation and irreversible inactivation of both the selectable and the muscle specific gene.

Analysis of transgenic mice carrying muscle specific genes revealed the following: 1. 145bp of the 5' flanking region of the rat skeletal muscle actin gene are sufficient to confer preferential expression in striated muscles. 2. The great variability in the tissue specific expression of CAT constructs was elliminated when the first intron was included. 3. In 2 transgenic strains the expression of the transgene was associated with a significant down-regulation of the endogenous gene. 4. The expression of the cardiac and skeletal muscle actin genes in the heart is tightly co-regulated.

Gene Transfer in Domestic Animals-III

F 010 **GENE TRANSFER, EXPRESSION AND INHERITANCE OF pRSV-TROUT-GH-cDNA IN FISH,** P.Zhang[1], C.M. Lin[2], M. Hayat[3], C. Joyce[3], L.I. Gonzalez-Villasenor[1], R. Dunham[3], T.T. Chen[1,2], and D.A. Powers[1,2], [1]Department of Biology, The Johns Hopkins University, Baltimore, MD; [2]Center of Marine Biotechnology, The University of Maryland, Baltimore, MD; [3]The Department of Fishery and Allied Aquaculture, Auburn University, Auburn, AL.

We are studying the mechanisms that control growth hormone (GH) gene expression in teleosts. A recombinant plasmid containing the LTR of RSV and trout GH cDNA was microinjected into fertilized carp eggs. Genomic DNA extracted from the dorsal fin of individual presumptive transgenic fish was analyzed by dot blot and Southern blot hybridization, using the LTR of RSV and trout GH cDNA as probes. Out of 380 presumptive transgenic fish analyzed, 20 individuals were found to contain RSVLTR-trout-GH-cDNA sequence in the genomic DNA. Expression of trout GH polypeptide was also detected in the transgenic fish. Although there was considerable variation in the sizes of the transgenic fish, they appeared to be 20% larger, on average, than their sibling controls. The largest transgenics were 50% greater than the mean size of the control fish. Some of the progeny derived from crosses between transgenic males and non-transgenic females were also found to carry the trout GH cDNA.

F 011 TECHNIQUES FOR THE DEVELOPMENT OF TRANSGENIC FISH : A REVIEW, Daniel Chourrout[1], René Guyomard[1] and Louis-Marie Houdebine[2], Laboratory of fish genetics[1], Unit of cell and molecular differentiation[2], National Institute for Research in Agriculture, 78350 Jouy-en-Josas, France.
Transgenic fish are of interest for biological studies and for genetic improvement in aquaculture. Advantages of fish over mammals are a usually high fertility and an external fertilization which permit the manipulation of numerous embryos. However, egg pronuclei have not yet been visualized and fish retroviral vectors are not available. The present review describes the production of numerous transgenic fish by injections of various gene constructions into the egg cytoplasm. Injection into the oocyte germinal vesicle is another possibility. Germ-line transmission has been demonstrated in the rainbow trout and the zebrafish. Preliminary observations of foreign gene expression in transgenic fish and in transfected fish cells are also presented.

F 012 CONTROL OF METALLOTHIONEIN GENE EXPRESSION IN THE RAINBOW TROUT, Lashitew Gedamu Per-Eric Olsson and Muhammad Zafarullah, Department of Biological Sciences, The University of Calgary, Calgary, Alberta, Canada, T2N 1N4
To study the regulation of metallothionein (MT) gene expression and to test the feasibility of MT gene promoter for the development of transgenic fish, we have cloned the rainbow trout MT-B gene. Its coding region comprises of three exons interrupted by three introns and like its mammalian counterpart(s) is very cysteine rich. In contrast with the mammalian MT promoter, its promoter region is A + T rich and lacks SP1 binding GC sequences. Deletion analysis of the 5'-flanking region placed upstream of there porter CAT gene and transfection of various constructs into the rainbow trout hepatoma (RTH) cells revealed that a 100 bp fragment was sufficient for induction by Cd and Zn. This fragment contains two copies of metal-responsive elements (MREs) resembling to the mammalian MREs. The promoter is also functional, but weakly active, in the human hepatoblastoma (HepG2) cell line, suggesting that an MT regulatory factor(s) is conserved in phylogenetically distant species like humans and fish. This gene is also expressed in a variety of trout tissues such as liver, kidney, spleen and gills in response to Cd, Cu and Zn injections. Following exposure to Cd and Zn, MT-B mRNA is accumulated in RTH and RTG-2 (gonad) cell-lines. However, it is not inducible in the chinook Salmon embryonic (CHSE) cells and DNA methylation appears to be involved in its regulation. We are currently studying its expression during development to investigate the potential of MT-B promoter in the production of trangenic fish.
Supported by MRC and AHFMR.

Embryo and Stem Cell Manipulation

F 100 EFFECTS OF HUMAN GROWTH HORMONE TRANSGENE ON FERTILITY AND REPRODUCTIVE AGING IN MICE. Andrzej Bartke, Kechun Tang, Paul Bain, J.S. Yun* and Thomas Wagner*. Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901 and *Edison Center, Ohio University, Athens, OH 45701.
Expression of mouse metallothionein I/human growth hormone (hGH) transgene in mice leads to female sterility which appears to be due to activation of hypothalamic dopaminergic neurons, suppression of endogenous PRL release and luteal failure (Bartke et al., J. Exp. Zool, in press). Most males are fertile, but exhibit elevated LH and suppressed PRL levels (Chandrashekar, Bartke and Wagner, Endocrinology, in press). In young transgenic females, the length of estrous cycle is significantly extended. Middle aged females do not cycle but can ovulate in response to PMSG/hCG injections. In transgenic females in which pregnancy was maintained by treatment with progesterone or PRL-secreting ectopic pituitary transplants, the lactational performance evaluated by litter growth and survival is unpredictable ranging from virtually absent to apparently normal. Cross-fostering experiments indicate that this is due to maternal factors. In middle-aged and apparently healthy males that cease to breed successfully, the libido (measured by mount latency during behavioral tests) is not suppressed and epididymal spermatozoa are fertile when used for artificial insemination. Sterility appears to be due to inability to intromit and ejaculate. Marked reduction in life span of these transgenic mice is associated with development of mammary tumors in females and gross (approximately 10-fold) enlargement of accessory reproductive glands in males. Supported by NICHD, HD20001 and HD09042.

F 101 MICROINJECTION OF THE BULL GROWTH HORMONE GENE INTO THE FERTILIZED EGGS OF RABBITS, Victor G. Bavin, Jozef Bulla, Pavel Babushik, Pavel Uhrin, Departmen of Genetics and Experimental Biology, Research Institute of Animal Production, 949 92 Nitra, Czechoslovakia.
Experiments with the aim to establish methods to obtain transgenic rabbits were made. Donors of fertilized eggs were females of following rabbit breeds: Nitriansky, New Zealand White, and the line Zobor. Approximately 19 hours after insemination the donors were slaughtered and from the prepared oviducts the eggs were flushed with the help of KREBS solution, warmed up to 37^{0}C, with the addition of 10 % fetal calf serum. About 1-2 pl. DNA solution in Tris-EDTA, pH7,5, were injected into the large pronucleus of each egg. About 500-1000 copies of the DNA linear fragment which contains promotor-regulator gene of metallothionein-I of mouse fused to the bull growth hormone gene (MTbGH), which we obtained from Dr. G. N. Yenikolopov, USSR, were injected into the eggs. Immediately after the injection the eggs were transfered surgically into the oviducts of the synchronized pseudopregnant rabbit recipients of Chinchilla breed. The recipients were transfered totally 91 fertilized eggs. Thirty five rabbits were born (38,2%), from them 25% died in first two days and the rest developed successfully. From all offsprings samples tissue of ear were taken in order to determine the frequency of integration of foreign gene by the method Dot, Blot hybridization of nucleic acids. Results of these analyses will be presented.

F 102 **TARGETING GENE EXPRESSION TO THE PNEUMOBRONCHIAL SYSTEM IN TRANSGENIC MICE,** **D.W. Bullock*, S. Damak*, F.J. DeMayo, and A.B. Mukherjee+.** Department of Biochemistry and Microbiology, Lincoln College, Canterbury, New Zealand*; Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; Human Genetics Branch, NICHD, Bethesda, MD 20892+

The gene for rabbit uteroglobin (UG) is expressed in the uterus, where it is induced by progesterone, and in the lung, where expression is constitutive or weakly regulated by glucocorticoids. Transgenic mice have been generated by pronuclear microinjection of cloned genomic UG DNA including 2.3 Kb of 5'-flanking sequence. Expression in mice was monitored by Northern analysis of total tissue RNA and by immunofluorescence of tissue sections stained with anti-UG antibodies. UG mRNA was detected in the lung and uterus, but not in liver, kidney, brain or spleen of transgenic mice. The level of UG mRNA in transgenic lung exceeded that in the rabbit, while uterine expression was weaker. Strong immunofluorescence was present in the bronchial and pulmonary alveolar epithelium but was not detectable in the endometrium. Thus injected DNA contains sequences capable of directing tissue-specific expression of the UG gene. The recent discovery of anti-inflammatory properties of UG makes these mice valuable models for studies of pulmonary inflammation. Use of the UG promoter region to target expression of other genes will provide other models of pulmonary disease.

F 103 OVEREXPRESSION OF THE VIMENTIN GENE IN TRANSGENIC MICE IS LINKED TO ABNORMAL LENS CELL DIFFERENTIATION AND CATARACT FORMATION. Y. Capetanaki, S. Smith, & S. Starnes, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
The unique expression pattern of the intermediate filament (IF) subunit vimentin motivates the desire to investigate its biological function during proliferation, differentiation and morphogenesis which remains largely unknown. Vimentin seems to interconnect the nucleus with the plasma membrane, possibly contributing in this structural way to the transport processes and communication taking place between the cell surface and the nucleus. Indeed, recent biochemical studies have shown high binding affinity of vimentin, and other non-epithelial IF subunits for nuclear constituents like lamin B, single stranded nucleic acids, particularly DNA, and core histones. However, to date, there is no evidence to support functional roles for any of the demonstrated in vitro structural interactions. To investigate the role of the IF protein vimentin in the normal differentiation and morphogenesis of the eye lens fiber cells, we generated transgenic mice bearing multiple copies of the chicken vimentin gene. The vimentin transgene was expressed in a correct tissue-specific pattern, the transcripts were properly processed to stable mRNAs which were efficiently translated to vimentin protein that could be post-translationally properly modified. In most cases, the vimentin transgene was overexpressed in the lenses of these animals, reaching up to ten times the endogenous levels. This high expression of vimentin interfered very strongly with the normal differentiation of the lens fibers. The normal fiber cell denucleation and elongation processes were impaired and the animals developed pronounced cataracts, followed by extensive lens degeneration.

F 104 ANTIFREEZE PROTEIN EXPRESSION IN TRANSGENIC SALMON, Peter L. Davies, Choy L. Hew, Margaret A. Shears and Garth L. Fletcher. Department of Biochemistry, Queen's University, Kingston; Research Institute, Hospital for Sick Children, Toronto; Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Canada.
Winter flounder are protected from freezing in ice-laden seawater by high concentrations of antifreeze proteins (AFP) that circulate in the blood. Atlantic salmon do not have the benefit of protection from these macromolecular antifreezes and will freeze at temperatures below -0.7°C. The lack of AFP in salmon poses a severe limitation to their aquaculture in sea-pens along the Atlantic coast. In an effort to improve the freezing resistance of this species we have transferred an AFP gene from winter flounder to salmon. The gene and flanking sequences (8kb) form a tandem repeat in flounder and should, therefore, contain the cis-acting sequences needed for regulation. Linearized AFP gene (1×10^6 copies) was microinjected through the micropyle of fertilized but non-activated salmon eggs. A small percentage of fingerlings hatched from these eggs were shown to have integrated copies of the AFP gene. Subsequently, two-year old fish from the same injection series were tagged and bled during the winter months. Immunoblot analysis of their sera indicated the presence of proAFP in 1% of the fish screened. Experiments are in progress to increase AFP levels and determine if expression is seasonally regulated in the transgenic salmon as it is in flounder. (Supported by MRC and NSERC, Canada).

F 105 **EXPRESSION OF A TRANSGENIC ANTI-CD8 IgM HEAVY CHAIN GENE IN THYMOCYTES INHIBITS EARLY T-CELL DEVELOPMENT. Hermann Eibel, Frank Brombacher & Georges Köhler, Max-Planck-Institute for Immunology, Stübeweg 51, 7800 Freiburg, West Germany.**
We created several transgenic mouse lines expressing either the secreted and membrane form (u^{s+}) or only the secreted form (u^s) of an anti-CD8 IgM heavy chain gene. The transgenes are expressed at normal levels in the B-cells. Expression also occurs in fetal as well as in adult thymocytes. Synthesis of the u^{s+} form inhibits CD8 surface expression on immature and mature thymocytes which leads to a 20 fold reduction of the $CD8^+4^+$ thymocyte population, expression of the u^s form has no effect. Our results show, that correct expression of CD8 during T-cell differentiation and its interactions as a cell adhesion molecule are essential for thymocyte development.

F 106 INTERRUPTION OF THE T LYMPHOCYTE DEVELOPMENTAL PROGRAM IN TRANSGENIC MICE BEARING AN *lck*-SV40 EXPRESSION CONSTRUCT, Katherine A. Forbush, Alex M. Garvin, Kristin M. Abraham, Andrew G. Farr, and Roger M. Perlmutter. Howard Hughes Medical Institute SL-15, University of Washington, Seattle, WA 98195. The *lck* gene encodes a *src*-family protein tyrosine kinase that is expressed specifically in lymphoid cells. We have used transcriptional regulatory sequences derived from the murine *lck* gene to direct the expression of SV40 large T antigen (SV40 T-Ag) in (C57BL6 X DBA/1) F_2 transgenic mice. The *lck*-SV40 transgene is expressed in peripheral lymphoid organs and at high levels in thymocytes, as is the endogenous *lck* gene. The transgene is also expressed ecotpically in renal tubular cells. Mice bearing the *lck*-SV40 construct have drastically reduced body weights and most die at an early age. Paradoxically, in light of the transforming activity of SV40 T-Ag, the thymuses of these animals are quite small, with thymocyte numbers reduced 10-100-fold as compared with littermate controls. Flow cytometric analysis reveals that SV40 T-Ag disrupts thymocyte development; surviving thymocytes are enriched for those with immature phenotypes, particularly $CD4^-CD8^-$ and $CD8^+CD3^-$. Cell lines derived from two animals that developed thymic tumors also had immature cell surface phenotypes. We conclude that the *lck* promoter is capable of driving the expression of heterologous gene constructs in a largely lymphocyte-specific fashion. Expression of SV40 T-Ag in thymocytes interrupts the normal T lymphocyte developmental program, permitting the partial dissection of thymocyte lineage relationships.

F 107 ATTEMPTED INTRODUCTION OF A NOVEL GENE INTO GOLDFISH THROUGH ELECTROPORATION, Eric M. Hallerman[1], Anthony J. Faras[2,3], Perry B. Hackett[3,4], Anne R. Kapuscinski[5], and Kevin S. Guise[1,3], [1]Department of Animal Science, [2]Department of Microbiology, [3]Institute for Human Genetics, [4]Department of Genetics and Cell Biology, and [5]Department of Fisheries and Wildlife, University of Minnesota, St. Paul, MN 55108.
Gene transfer in goldfish, Carassius auratus, was attempted using novel methodology. Although electroporation, the introduction of foreign DNA into cells by means of electrical pulse, has proven useful for transformation of bacterial, plant, and mammalian cells, it has never been utilized upon fish cells. Attempts were made to transfer a marker gene conferring resistance to the antibiotic chloramphenicol into one cell-stage goldfish embryos. Results to date have proven negative, which is attributed to the very small voltages which had to be applied using a capacitor-discharge power source. Integrated circuit-based power sources may prove more useful in production of transgenic fish.

F 108 ALLELIC EXCLUSION IN MUTANT Ig-HEAVY CHAIN TRANSGENIC MICE.
Antonio Iglesias, Marinus Lamers, Manfred Kopf and Georges Köhler, Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG:
Expression of immunoglobulin μ or δ chains in transgenic mice leads to inhibition of rearrangement of endogenous V_H gene segments and thus results in almost exclusive expression of transgenic heavy chains in B-cells of these mice. It has been shown that only the membrane, but not the secretory μ-chain inhibits rearrangement. In order to further define the structural requirements of μ-chains in this process, we produced transgenic mice with μ-constructs lacking different constant domains (Cμ1 or Cμ2 or Cμ3 and Cμ4). So far only the Cμ1-less construct appears to affect transgenic lymphoid development: the mice show a 10-20 fold reduction of B-cells in spleen and bone marrow. Depending on the genetic background, 30% to 80% of the B-cells left in transgenic spleens coexpressed endogenous and mutant μ-chains. The population of B-cells expressing only transgenic H-chains, which makes up the majority of splenic B lymphocytes in μ- or δ-transgenic mice, is missing in Cμ1-less transgenic mice. Also, in B-cell hybridomas from these mice endogenous V_H loci can be found that are arrested in an unrearranged state. Additionally, only the intact μ-chain, but none of the mutant proteins, was able to induce rearrangement of the V_K-J_K locus upon gene transfer and expression in an Abelson-transformed pre-B-cell line. These findings suggest that the Cμ1 domain is dispensable for inhibition of rearrangement at the heavy chain locus, but essential for B-cell muturation.

F 109 ANTIGEN EXPRESSION AND IMMUNE RESPONSE IN SINGLE AND DOUBLE HLA-CLASS-1 TRANSGENIC MICE (TGM). Pavol Ivanyi and Femia Kievits. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Department Experimental and Clinical Immunology, University of Amsterdam, Plesmanlaan 125, 1006 AD Amsterdam, The Netherlands.
Cell membrane expression of HLA Class 1 molecules in TGM is clearly dependent on the presence of a second transgene: human beta-2-microglobulin. However, HLA Class 1 antigens can be detected on cell membranes also in association with mouse b2m, albeit at lower levels. Antibodies and cytotoxic lymphocytes can be induced by both single and double HLA Class 1 TGM lymphocytes and first anti-TGM McAb's were isolated. Finally, HLA molecules in both single and double TGM cell membranes were able to present foreign antigen to the T-cell receptors. Taken together, these data show, that in B27 TGM mice the HLA molecules are fully functional. Hence these mice can be used for studies on the role of HLA-B27 in disease associations, under the presumtion that B27 molecules themselves are involved in the pathogenesis. Ankylosing spondylitis and acute anterior uveitis appaer as diseases of "first choice" for these studies.

F 110 **APPROACHES TO THE TARGETED MUTAGENESIS OF THE PIM-1 GENE IN THE MOUSE GERM LINE**, Peter W. Laird, Roanna Ueda, Els Hulsebos and Anton Berns, Division of Molecular Genetics, The Netherlands Cancer Institute and the Dept. of Biochem. of the UVA, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
The *pim-1* gene encodes a protein kinase with an as yet unknown substrate and function. The gene is activated by proviral insertion in about half of the cases of MoMuLV-induced T-cell lymphomas in the mouse. We are attempting to mutagenize the *pim-1* gene in the mouse germ line to gain insight into the normal function of this gene. We are currently comparing different strategies for the targeted mutagenesis based on homologous recombination of the *pim-1* gene with engineered genomic DNA fragments introduced into the cell. These strategies include selective and non-selective procedures in mouse embryonic stem cells as well as direct micro-injection of mouse zygotes followed by screening of fostered progeny. The recombination frequencies in micro-injected zygotes can be checked by Polymerase Chain Reaction analysis of blastocyst DNA. The non-selective procedures are based on the micro-injection of a 15-kb genomic DNA fragment containing a 23-bp disrupting linker 1.5 kb from one end of the fragment. Recombination with the endogenous *pim-1* gene can be detected in small numbers of cells by Polymerase Chain Reaction across this 1.5 kb. Data on the frequency of homologous recombination will be presented.

F 111 Molecular cloning and microinjection of a atlantic salmon growth hormone gene.
Rune Male, James Lorens, Ivar Lossius, Audun H.Nerland, Wenche Telle., and Geir Totland. Marine Genetics A/S, Center of Biotechnology, University of Bergen, Norway.
A genomic library from atlantic salmon (**Salmo salar**) was constructed and screened with oligonucleotide probes derived from conserved growth hormone sequences. Restriction enzyme analysis of several clones revealed two different growth hormone genes, designated sGH I and sGH II. A pituitary gland cDNA library was constructed and five GH-cDNA clones characterized, all representing the sGH I gene. The genomic and cDNA sGH I nucleotide sequences demonstrated strong homology to all known salmonoid genomic and cDNA GH sequences.
More than 1000 eggs were microinjected with a 6 kb genomic sGH I DNA fragment during the 1987 spawning season. The injected DNA fragment contained a short terminal cloning vector sequence which will be utilized as a unique primer recognition site in a polymerase chain reaction assay to identify fish harboring the injected gene.

F 112 ANTISENSE RNA PRODUCTION IN TRANSGENIC MICE, M. Idrees Munir, Belinda J.F. Rossiter and C. Thomas Caskey, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

The inhibition of gene expression in eukaryotic systems by antisense RNA may occur by the binding of sense and antisense message in the cytoplasm, preventing the translation of a specific mRNA, or by hybridization of the two species in the nucleus, preventing the processing or transport of the targeted message. A 5' genomic fragment from the mouse HPRT gene, containing the first exon, part of the first intron and the natural promoter, was inserted in the reverse orientation into an expression vector containing the inducible mouse metallothionien-1 promoter and the human growth hormone 3' poly A addition signal. Transgenic mice carrying this construct produced antisense RNA only in the central nervous system but reproducible inhibition of HPRT activity in this tissue was not observed. Further transgenic mice were also generated from the same construct but lacking the HPRT natural promoter in the insert. Three of these mice showed antisense expression in heart and liver and are currently under investigation.

F 113 THE ANALYSIS OF STERILITY IN TRANSGENIC MICE INDUCED BY INSERTIONAL MUTAGENESIS, Theodore Pellas, Maria Marone, Banu Ramachandram, Kiran Chada, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854

Introduction of foreign DNA into the fertilized mouse egg may lead to the disruption of a developmental locus. One transgenic mouse line for a foreign globin gene insert gave rise to a recessive mutation that led to sterility in both males and females. The defect in female mutant mice mimics primary ovarian failure or premature menopause in women, and Sertoli cell only syndrome in men. Homozygous females are sterile, yet cycle normally until 5 months of age when they prematurely cease cycling due to lack of developing follicles. Homozygous males copulate with females and form a normal vaginal plug, but sperm counts are 1/10 of normal, and thus are subfertile. It is proposed to define the phenotype of this mutation by analyzing the gonads at different developmental stages by histopathological examination. Most importantly, this method of insertional mutagenesis allows one to clone the normal locus which has been disrupted by using the foreign DNA as an initial probe. It is therefore proposed that this gene will be cloned in order to ultimately define the function of this gene product in the process of mammalian germ cell development.

F 114 THE PRODUCTION OF CHIMERIC CHICKS BY EMBRYONIC CELL TRANSFER, J.N. Petitte and R.J. Etches, Dept. of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Early embryonic cells from a line of Barred Plymouth Rocks were injected into the blastocoele of unincubated Dwarf White Leghorn embryos. Donor blastoderms (5) were removed from the egg, dissected free of adhering yolk, placed in Medium 199, and washed twice to remove any remaining yolk. The embryonic cells were dispersed with 0.25% trypsin/.04% EDTA at 37°C for 10 minutes. Trypsin was removed by washing twice with Medium 199 and the pellet was resuspended in 1 ml of medium. Approximately 14,000 cells/blastoderm were isolated with greater than 95% viability. A 0.5 cm window was made in the equatorial plane of the recipient egg over the region of the blastoderm. Transfer was accomplished by microinjection of 200-500 cells into the blastocoele of the recipient embryo. The window was sealed with a coverslip and paraffin wax. From 10 eggs, four died by day 7, three died by day 17-18, and three viable chicks hatched. Of the three embryos which died at day 17-18, one was clearly chimeric as evidenced by black down on the head and back. Of the three viable chicks, one exhibited black down on the face and head. This technique allows the production of chimeric chicks and may be useful in the development of transgenic poultry.

F 115 MALE HYPOGONADISM IN TRANSGENIC MICE CARRYING A HGR PROMOTER/c-myc FUSION GENE, F. Pothier, M.V. Govindan, Y. Tong, G. Pelletier, Molecular Endocrinology Laboratory, CHUL Research Center, Laval University Medical Center, Québec, Canada, G1V 4G2. Our group has isolated the promoter region of the human glucocorticoid receptor gene (hGR) from the λEMBL-3 human genomic library. The sequence of the hGR promoter reveals that it does not contain a "TATA box" and has a very high "C+G" content. Gene transfer studies with hGR-CAT chimeric plasmids into CV-1 cells showed that the hormonal regulatory sequences of hGR are contained within a 4.0 EcoRI-XbaI fragment. It also appears that the hGR is down regulated by glucocorticoids. We have constructed a chimeric plasmid using the 4.0 kb EcoRI-XbaI fragment of the hGR promoter fused to the 4,6 kb c-myc oncogene fragment. The production of transgenic mice with the hGR/c-myc gene would allow us to look at the regulation of the receptor gene in vivo by monitoring the expression of c-myc. At the same time, we were interested in the complementation of the c-myc oncongene regulated by the hGR promoter in different tissues. We present premilimary results obtained from five different founders (3 males, 2 females). Although the characterization of the expression of the transgene is not completed, we can detect its effects in at least two tissues: all the transgenic males (founders and offsprings of female founders) are sterile and show atrophied testis and prostate. Histological analysis of the testis indicates the presence of Leydig cells with underdeveloped seminiferous tubules. We are producing trangenic mice with the same hGR promoter linked to CAT gene to determine if the regulatory sequences of hGR alone are responsible for these effects.

F 116 EPISOMAL ELEMENTS IN TRANSGENIC MICE. M. Rassoulzadegan, P. Léopold, J. Vailly, A. Blangy & F. Cuzin, - Unité 273 INSERM, Centre de Biochimie, 06034 Nice, France. We reported previously (1) the establishment of a series of transgenic mouse strains which stably maintained autonomous circular DNA molecules in low copy numbers. Their presence in the germ line and high frequency of transmission indicated that they segregate efficiently at meiosis. These effects were the result of the co-injection of plasmid pPyLT1, encoding the large T protein of polyoma virus, and of a minute amount of another plasmid, p12B1, which carries a 345 BP mouse DNA sequence with a binding site for large T (2). Five of these twelve transgenic strains maintain unrearranged p12B1 DNA molecules, while the other plasmids appear as "patchwork" structures with a juxtaposition of parts from p12B1 and from pPyLT1, and of mouse sequences of unknown origin. The mouse DNA in p12B1 and in one of the patchwork plasmids which was completely sequenced, p12B2, are not homologous, but they both include nucleotide boxes corresponding to the consensus for murine ARS sequences (3) and to the consensus for yeast centromere elements CDE1 and CDE3 (1). Gel retardation assays evidenced a binding site for a protein present in nuclear extracts of cultivated mouse cells, corresponding to, or overlapping with the CDE1 element. In addition, both the p12B1 and p12B2 mouse sequences exhibit inverted repeats of various lengths. Transient replication of p12B1 DNA could be evidenced upon transfection in cultivated mouse cells. Depending on a series of experimental conditions which are presently under study, injection in mouse eggs of p12B1 and of combinations of p12B1 and pPyLT1 DNA led either to the maintenance of free circular molecules or to a highly efficient integration into host sequences. The latter phenomenon was also observed in cell culture upon selection for a linked neo resistance gene. The occurence of non-random recombination events was suggested by the observation that in independent sub-lineages of one of the plasmid-bearing transgenic families, apparently identical and complex rearrangements of the transgene occured after 5 to 6 generations of purely autonomous transmission. Ref.: (1) Rassoulzadegan et al., 1986, Cell, 46:513; (2) Léopold et al., 1987, Cell, 51:885; Holst et al., 1988, Cell, 52:355.

F 117 MICROINJECTION AND SUCCESSFUL TRANSFER OF THE BOVINE GROWTH HORMONE GENE INTO THE NORTHERN PIKE, ESOX LUCIUS. Schneider, J.F.[1,3], Hallerman, E.M.[2], Yoon, S.J.[2], He, L.[4], Myster, S.H.[1], Gross, M.[4], Liu, Z.[5], Zhu, Z.[6], Hackett, P.B.[1,5], Guise, K.S.[1,2], Kapuscinski A.R.[4], Faras, A.J.[1,3] [1]Institute for Human Genetics, Departments of [2]Animal Sciences, [3]Microbiology, [4]Fisheries and Wildlife, and [5]Genetics and Cell Biology, University of Minnesota, St. Paul MN 55455. [6]Institute for Hydrobiology, Academia Sinica, Wuban Hubie, Peoples Republic of China.

Aquaculture relied on genetic breeding programs in the past for improvements in stock traits of fish. Although some limited success has been observed with certain species of fish, little if any of the traditional genetic breeding programs have been successfully applied to sportfish. For this reason we have embarked on a series of studies to transfer relevant genes into sportfish species employing contemporary molecular biological procedures. In this communication we report the successful transfer and expression of the bovine growth hormone gene (bGH) into newly fertilized eggs of the northern pike, Esox lucius. The cDNA sequence for bGH was inserted into a casette vector utilizing the Rous sarcoma virus upstream LTR as a promoter. This construct was linearized and microinjected into freshly fertilized eggs 20-30 minutes post fertilization. DNA was extracted from juvenile fish fin clips and analyzed by Southern blot hybridization. Approximately 20% of the microinjected fish contained bGH DNA. RNA was prepared from the anterior portion of 2 month old fish and analyzed by slot blot hybridization. Approximately one third of the DNA-positive microinjected fish also expressed bGH specific RNA. Surprisingly, some individual fish which lacked DNA within their fins expressed RNA within their bodies suggesting mosiacism of the microinjected bGH DNA. Blood growth hormone levels and fish growth are presently being measured. Preliminary data indicates the fastest growth rate observed among siblings occurs among fish positve for the injected bGH gene.

F 118 ESTABLISHMENT OF CANINE MUSCLE-TYPE PHOSPHOFRUCTOKINASE DEFICIENCY AS A MODEL FOR SOMATIC CELL GENE REPLACEMENT THERAPY, Hansell Stedman, Jim Sylvester, Dirke Pette, & Urs Giger, U. of Penn., Philadelphia, PA 19104
By virtue of its unique histogenesis, skeletal muscle represents a favorable target for somatic cell gene replacement therapy. It is now well established in rodent systems that transplanted myoblasts can migrate substantial distances from their injection sites, and contribute to muscle fiber growth and regeneration. We wish to explore this approach in an outbred mammalian species, and ultimately couple in vitro genetic manipulations to emerging techniques in cell transplantation. Therefore, we have undertaken the multidisciplinary study of an autosomal recessive phosphofructokinase (PFK) deficiency in the dog. The clinical manifestations of PFK deficiency in this species, and human patients, include a hemolytic disorder and metabolic myopathy. Skeletal muscle PFK activity is less than 2% of control levels and the defect is readily demonstrated by histochemical techniques and in vivo by 31P-magnetic resonance spectroscopy. Polyclonal antisera specific for mammalian M-type PFK detect less than 5% of control levels of a normal sized PFK polypeptide on western blots of PFK-deficient muscle preparations. The only mammalian PFK gene cloned to date is 17 kb in length with 2.7 kb of coding sequence. We have cloned a homologous 2.5 kb cDNA fragment from human fetal skeletal muscle. Use of this cDNA in northern blotting experiments reveals a normal sized (2.7 kb) transcript at normal abundance in PFK deficient muscle. We interpret these finding to indicate the presence of a point mutation in the PFK coding sequence. Current experiments are directed towards the identification of the site of the mutation and the development of muscle transplantation and somatic gene replacement therapy in this disease model.

F 119 DEVELOPMENTAL ACTIVATION OF THE TAT GENE INVOLVES SYNERGISM OF ITS TWO ENHANCERS, A.F.Stewart,E.Schmid,W.Schmid,F.Weih,M.Boshart and G.Schütz, Institute for Cell and Tumor Biology, German Cancer Research Center, Heidelberg D-6900, West Germany. The rat TAT (tyrosine aminotransferase) gene is constitutively expressed in the adult liver and in H4 hepatoma cell lines. Its transcriptional activity can be stimulated more than fivefold by either glucocorticoids or cAMP and these effects are additive. The glucocorticoid and cAMP responses are mediated through enhancer elements 2500 and 3600bp, respectively, upstream of the start site of transcription and are characterised by the presence of DNAse1 hypersensitive sites. We have been examining the state of the gene in fetal liver and in an H4 cell line that contains the human Tse-1 (tissue specific extinguisher). We find in both cases that the quiescent gene is responsive to either glucocorticoids or cAMP as reflected by the rapid induction of the respective hypersensitive sites. Neither inducer alone is sufficient to induce either the onset of transcription or the hypersensitive site that characterises the active promoter. However administration of both inducers together is sufficient. We reason that the developmental onset of transcription of TAT is achieved by a hormonally responsive, two-component switching of the poised gene.

F 120 SOMATIC GENE THERAPY OF A MOUSE ENZYME DEFICIENCY USING A RECOMBINANT ADENOVIRUS. Jean-François Chasse[1], Pascale Briand[1], Leslie Stratford[2], Massimo Levrero[2], and Michel Perricaudet[2]. 1.Hopital Necker, 75743 Paris Cedex, France. 2. Institut Gustave-Roussy 94805 Villejuif Cedex France.
We have constructed and injected a recombinant adenovirus which harbors the rat ornithine transcarbamylase gene (OTC) under the control of a viral promoter into the spf-ash OTC mutant strain of mice. This strain is characteristized by a reduction in the amount of OTC activity, OTC protein, and specific OTC mRNAs resulting in hyperammonemia, pronounced orotic aciduria, growth retardation, and sparse fur until weaning. Most of the injected animals were found to produce one month following the injection an enhanced liver OTC activity as compared to spf-ash control animals, and thus whatever the injection route chosen (injection into the liver or intravenously into the tail). The synthesis of OTC was also accompanied by a reduction of the orotic aciduria showing that the injected sequence is able to restore the unpaired OTC métabolism. Moreover, the appearance of fur before weaning, was observed in the animals producing the highest levels of OTC, Thus, adenovirus can constitute a new tool in the development of somatic gene therapy.

F 121 A NOVEL DWARF MUTATION CAUSED BY TRANSGENIC INSERTION, Makoto Taketo and Amy L. Greene, The Jackson Laboratory, Bar Harbor, ME 04609

In order to study the tissue specificity of a cellular enhancer that is active in embryonal carcinoma (EC) cells [Taketo, M. & Tanaka, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**:3748-3752], we generated transgenic mice that carry this enhancer linked to a marker gene. During the course of breeding them into homozygous strains, we have found one of the transgenes has caused a dwarf phenotype. This is an autosomal recessive mutation with Mendelian inheritance and linked to the transgene. Body weight of the affected animals ranges 60-65% of the littermates. Although we lost some animals before weaning, most dwarf animals have survived well up to more than six months so far. Electron microscopical examination of the anterior pituitary of the affected animals has revealed degeneration of a particular cell type that contained hormone granules. Except for being small, there has been no other signs of complications such as disproportionate body development, unusual facial appearance or cleft palate etc. Compared with already described mutations that cause dwarfism, this mutation is distinctly different from little (*lit*), Snell dwarf (*dw*), or Ames dwarf (*df*). We are in the stage of testing the allelism with these mutations and mapping the gene locus by *in situ* hybridization of the chromosomes. The integration site of insertion mutation is being cloned.

F 122 REGULATION OF TWO CLOSELY RELATED MURINE α-CRYSTALLIN GENES, Eric F. Wawrousek, Robert A. Dubin, Ana B. Chepelinsky, Joan B. McDermott and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892

αA- and αB-crystallin are closely related (56% similarity) structural proteins in the vertebrate ocular lens. Northern blot analysis revealed αA-crystallin mRNA exclusively in lens of mice. αB-crystallin mRNA was present in lens and, in lesser amounts, in heart, skeletal muscle, lung, kidney and brain. αA-crystallin promoter-CAT fusion genes, or an αB-crystallin minigene were introduced into the germ lines of mice. Several lines of transgenic mice containing αA promoter sequences -111/+46 or -88/+46 expressed CAT exclusively in the eyes. The αA-CAT transgenes were activated in lens during embryonic development at approximately the same time as the endogenous αA gene and transcription initiated at the same site as in the endogenous αA gene. Three lines of mice carrying an αA -34/+46 CAT construct did not express CAT, suggesting that regions between -34 and -88 are essential for αA promoter function in mouse lens. Transgenic mice containing an αB-crystallin minigene construct (consisting of 666 bp of 5' flanking sequence and 75 bp of exon 1 fused to 300 bp of exon 3 with 2.4 kb of 3' flanking sequence) synthesized minigene mRNA in tissues expressing the endogenous αB gene (except possibly not in brain). Primer extension analysis demonstrated that transcription of the minigene in these tissues initiated at the same site as the endogenous αB gene. Both αA- and αB-crystallin had been presumed to be exclusively lens structural proteins but clearly αB also has non lens functions.

F 123 ENHANCED GROWTH OF TRANSGENIC FISH CONTAINING SOMATOTROPIN GENES, Grigory N. Yenikolopov[1,2], Alexei O. Benyumov[2], [1]Institute of Molecular Biology, USSR Acad. Sci., Moscow, USSR, [2]Department of Embryology, Moscow State University, Moscow, USSR

Plasmid DNA containing somatotropin hormone (STH) genes of various origin was microinjected into the cytoplasm of fertilised loach (M. fossilis) ova. The recombinant constructions contained, apart from cDNA copies of STH genes, the metallothioneine gene (MT) promoter and various splicing/polyadenylation signals. Over 50% of the embryos reached the hatching stage after the DNA injection (52-63% for different concentrations of DNA) with embryos dying mainly at the stage of late organogenesis. Larvae grew up to the juvenile stage (17 weeks) were kept in a 0.05 mM $ZnSO_4$ solution for 5.5 weeks in order to activate the MT-promoter. Southern blot analysis demonstrated that about 40% of the survived fish contained the integrated DNA sequences (2-30 copies per genome), which have not undergone any essential rearrangements upon microinjection and integration.

The phenotypic manifestation of the STH gene action in transgenic fish was estimated by statistically comparing the body length and mass. The transgenic specimens were almost twice larger than the control ones (noninjected and nontransformed)-1562 mg vs 852 mg mean mass.

S_1-analysis of the RNA demonstrates correlation of the amount of specific RNA molecules and the accelerated growth of individual specimens and proper utilization of the transcriptional start points in transgenic fish.

The results of similar experiments on zebrafish and trout will be presented.

F 124 PRODUCTION OF TRANSGENIC MICE FOR ANALYSIS OF MAMMARY SPECIFIC GENE EXPRESSION, Heng-Cherl Yom[1], Jan K. Heideman[2], Robert D. Bremel[1] and Neal L. First[2], Department of Dairy Science[1] and Department of Meat and Animal Science[2], University of Wisconsin, Madison, WI 53706

We have produced transgenic mice for the study of gene expression in mammary gland. A 3.4 kbp linear fragment containing the mammary specific MMTV promoter, bacterial chloramphenicol acetyl transferase (CAT) gene, SV40 early splice region and polyadenylation site was excised from pMSG-CAT. This fragment was isolated on 1% agarose gel, purified by Gene-Clean kit, and diluted to a concentration of 2-4 μg/ml in 10 mM Tris-Cl and 100 μM EDTA at pH 7.4. Seven to ten week-old C57B6 X DBA2J F1 (B6D2) female mice were superovulated and mated with ICR or B6D2 males to provide zygotes for pronuclear injection of the 3.4 kbp pMSG-CAT fragment. Of 226 injected zygotes, 118 progressed to normal-appearing 2-cell embryos, and were transferred into the oviducts of 4 ICR psuedopregnant females. Eleven pups were born, of which 3 were transgenic as determined by dot blots of tail DNA using nick translated CAT gene probe. We plan to use these transgenic mice as models to examine the feasibility of application of transgenic technology in lactating farm animals in which foreign proteins can be secreted as milk proteins.

Choice of Vectors in Transgenic Models

F 200 TRANSGENES BASED ON THE BOVINE VASOPRESSIN GENE ARE EXPRESSED WITH BOVINE SPECIFICITY IN MICE, Ang Hwee Luan, David Carter and David Murphy, Neuropeptide Laboratory, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

In addition to its classical hypothalamic sites of expression, the vasopressin (VP) gene is expressed in some peripheral tissues. Different species have different patterns of peripheral expression. For example VP is expressed at high levels in the bovine ovary but not in the mouse ovary. Two transgenes based upon the bovine vasopressin gene have been introduced into mice: 1.25 kb of VP 5' upstream sequences promoting the expression of SV40 large T-antigen and a 13.4 kb bovine genomic segment containing the coding seqences for VP and 8 kb of 5' upstream sequences. Both transgenes are expressed with bovine specificity in the murine ovary. All the information neccesary for ovary specific expression must therefore be contained within 1.25 kb of VP 5' upsream sequence. These transgenic mice are currently being used to study the function and regulation of ovarian VP and may prove to be useful models for an aspect of ruminant reproductive physiology that is difficult to approach experimentally.

F 201 INTERFERON-DEPENDENT EXPRESSION OF THE ANTI-INFLUENZA VIRUS PROTEIN MX IN TRANSGENIC MICE. Heinz Arnheiter, Stephen Chang, Ellen Meier, Susan Skuntz, Mathieu Noteborn* and C. Weissmann*. Laboratory of Molecular and Viral Pathogenesis, NINCDS, NIH and *Institute for Molecular Biology I, University of Zuerich, Switzerland.

Specific resistance of $\underline{Mx}^+$ mice to influenza virus is due to intracellular accumulation of the interferon alpha/beta induced $\underline{Mx}^+$ protein. In order to transfer influenza virus resistance to $\underline{Mx}^-$ animals, an $\underline{Mx}$ cDNA was placed either under control of the constitutive SV40 early enhancer/promoter or the interferon responsive $\underline{Mx}$ promoter, and the constructs were injected into $\underline{Mx}^-$ one-cell mouse embryos. None of three tested lines of SV40-Mx transgenics expressed Mx protein. Of nine founders obtained with the $\underline{Mx}$ promoter, six expressed Mx protein at high levels in their peritoneal macrophages provided these cells were exposed to interferon, one expressed at a low level, and two did not express. Three of the founders expressing at high levels, the weakly expressing and the two non-expressing founders were bred. Peritoneal macrophages of their transgenic F1 progeny were tested for interferon dependent Mx protein expression and susceptibility to infection with influenza virus in culture. Macrophages from mice of the three strongly expressing lines were resistant to influenza virus, macrophages from mice of the weakly expressing line were semi-resistant, and macrophages from mice of the two non-expressing lines were susceptible.

F 202 GERMLINE TRANSMISSION OF EXOGENOUS GENES IN THE CHICKEN FOLLOWING MICROINJECTION OF EMBRYOS WITH A NON-REPLICATING RETROVIRUS VECTOR, Robert A. Bosselman, Rou-Yin Hsu, Tina Boggs, Sylvia Hu, Joan Bruszewski, Susan Ou, Lee Kozar, Frank Martin, Cal Green, Frederick Jacobsen, Margery Nicolson, Joseph A. Schultz, Kenneth M. Semon, William Rishell, and R. Gregory Stewart, Amgen Inc., 1900 Oak Terrace Lane, Thousand Oaks, CA 91320 and Arbor Acres Farm, Inc., Marlborough Road, Glastonbury, CT 06033
Microinjection of non-replicating REV vectors into unincubated chicken embryo blastoderms leads to infection of both somatic and germline stem cells. About 8% of male birds hatched from injected embryos contained vector DNA in their semen. All positive males tested passed vector sequences on to their progeny. Analysis of G1 offspring shows that gonads of Go male birds are mosaic with respect to insertion of vector provirus. We conclude that primordial germ cells present in the unincubated chicken embryo blastoderm are susceptible to REV infection.

F 203 UROKINASE-TYPE PLASMINOGEN ACTIVATOR: TISSUE-SPECIFIC AND DEVELOPMENTAL GENE REGULATION IN TRANSGENIC MICE.
Florence Botteri[1], Herman van der Putten[2], and Yoshikuni Nagamine[1].
1: Friedrich Miescher-Institute, P.O.Box 2543, 4002 BASEL, Switzerland;
2: Ciba-Geigy Ltd., P.O.Box 2543, 4002 BASEL, Switzerland.
Urokinase-type plasminogen activator (uPA) is a serine protease involved in a number of physiological processes comprising cell migration and tissue remodeling, e.g. mammary gland involution, blastocyst implantation in utero, ovulation, spermatogenesis, inflammatory reactions and tumor cell metastasis. The diversity of biological processes in which uPA plays a role is reflected in the wide range of cell types expressing the uPA gene either transiently or constitutively. However, dramatic variations are observed when comparing the levels of uPA gene expression in the same tissue of different species (e.g. mouse and pig). These intriguing results are presently not explicable. For better understanding of the physiological functions of uPA, it is most important to identify the cell types that synthetise and release uPA. Some information is available on tissue-specific and developmental expression of mouse and pig uPA genes. To initiate detailed studies on tissue-specific uPA gene expression, we generated transgenic mice. These harbor a chimeric gene composed of the mouse or pig uPA promoter linked either to the gene encoding the bacterial enzyme chloramphenicol acetyltransferase (CAT) or to a modified gene encoding the E.Coli beta-galactosidase. The expression of these chimeric genes will allow a detailed study of tissue-specific expression of the mouse and pig uPA promoter throughout mouse development.

F 204 THE EXPRESSION OF A METALLOTHIONEIN-OVINE GROWTH HORMONE FUSION GENE IN TRANSGENIC MICE DOES NOT IMPAIR FERTILITY BUT RESULTS IN PATHOLOGICAL LESIONS IN THE LIVER, Jacqueline M. Orian, Chee Seong Lee, Linda M. Weiss and Malcolm R. Brandon, Department of Veterinary Preclinical Sciences, The University of Melbourne, Parkville, Victoria, 3052, Australia. The physiological effects of high serum levels of ovine growth hormone were studied in three generations of transgenic mice carrying a metallothionein 1-ovine growth hormone fusion gene. Livers of mice expressing ovine growth hormone were enlarged, irrespective of the level of serum ovine growth hormone detected. In mice expressing high levels of ovine growth hormone, direct measurements of hepatocytes in liver sections revealed that cell and nuclear size were abnormally large. Hepatocytes of different transgenic mice varied from 1.4 to 2.2 times normal size and hepatocyte nuclei varied from 1.7 to 2.4 times normal size. In addition, intranuclear inclusions were observed in hepatocytes of transgenic mice and their presence was always associated with high serum levels of ovine growth hormone. In contrast to female transgenic mice containing mouse metallothionein 1-human, rat or bovine growth hormone fusion genes female mice containing the metallothionein-1 ovine growth hormone fusion gene were fertile and their pituitary glands showed synthesis of growth hormone.

F 205 MYELIN SPECIFIC EXPRESSION OF AN ATTENUATED DIPHTHERIA TOXIN GENE IN TRANSGENIC MICE EXPRESS A SHIVERING PHENOTYPE, S.Chang[1], L.Harrington, H.Arnheiter, L.Hudson, A.Bernstein, M.Breitman, and R.Lazzarini, [1]Viagene, Inc. San Diego, CA 92121. Myelin basic protein (MBP) is one of the more abundant proteins in the central and peripheral nervous system. This protein is believed to be important in the induction of myelination, deposition of the myelin sheath and wrapping and compaction of the nerve axon. Deletion of MBP function in the shiverer mouse mutation results in a specific defect in CNS myelination that results in an action tremor, progressive convulsions and death in 50-100 days after birth. We have produced transgenic mice carrying a mouse MBP promoter diphtheria toxin gene to begin analysis of myelin basic proteins role in the central and peripheral nervous system. Two constructs were made containing the mouse MBP promoter with 6.5 kb of 5' flanking genomic DNA and either the wild type or attenuated diphtheria toxin gene. These constructs contain no introns or splice junctions within the coding regions. No animals were obtained that contained the MBP wild type diphtheria construct. Two animals were obtained that contained the MBP attenuated toxin gene. Both founder animals display a tonic tremor phenotype. The founder male was determined to be sterile while the female is fertile. So far, transmission of the transgene is at a low frequency. Assessment at the cellular and molecular level of the shaking phenotype is currently underway.

F 206 EXPRESSION OF BOVINE GROWTH HORMONE GENE IN TRANSGENIC CHICKENS, Howard Y. Chen; Ellen A. Garber; Charles I. Rosenblum; Joyce E. Taylor; John J. Kopchick; Roy G. Smith; James Smith* and Edward O. Mills*, Department of Growth Biochemistry and Physiology, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065 and Hubbard Farms*, Walpole, NJ 03608. Bovine growth hormone (bGH) gene was linked to the murine metallothionein-1 promoter and inserted into a nonpermuted high titer Rous Sarcoma viral vector in an orientation opposite to that of viral gene transcription. High titers of virus ($10^6 \sim 10^7$/ml) was isolated 5 days after transfecting the recombinant DNA into chicken embryo fibroblasts. Transgenic chickens were generated by injecting recombinant virus into fresh fertile Leghorn eggs under the blastoderm. The chickens were analyzed for the presence of 1) virus in the day-old meconium samples, 2) bGH and viral sequences in the DNA of red blood cells, 3) bGH protein in the serum. Radioimmunoassay results showed bGH levels ranging from 10 to 280 ng/ml can be detected in various chickens. Two male chickens expressing high levels of bGH (approx. 100 ng/ml) had longer legs (20 cm) at 21 weeks of age than the control chickens (18 cm). However, this effect was not observed with other chickens expressing lower levels of bovine growth hormone.

F 207 ANALYSIS OF TRANSGENIC MICE WITH HUMAN PAPILLOMAVIRUS SEQUENCES, Kong-Bung Choo, Lip-Nyin Liew and Winston T.-K. Cheng Department of Medical Research, Veterans General Hospital, Taipei & Department of Animal Husbandry, National Taiwan University, Taipei, Taiwan, Republic of China.
Human papillomavirus (HPV) is an ubiquitous infection agent found in many body sites of the humans. Some of the HPV types, e.g. HPV16 & 18, are assocaited with carcinomas of the oral cavity and genital tract, particularly cervical carcinomas. We have constructed 8 tg mouse lines carrying the entire HPV16 or HPV18 genome, or the E6/E7 genes of HPV16 which are thought to be involved in the transformation of the host cells. Physical analysis indicates that one of the lines carries three segregatable transgenic chromosomes with tandem duplications while another also carries multiple integrations but probably in the same chromosome. Two of the lines analysed are mosaics while other cases carry single integrations with tandem duplication. Results on the analysis of HPV gene expression will be presented.

F 208 REGULATION OF ANGIOTENSINOGEN GENE EXPRESSION IN TRANSGENIC MICE, William M Clouston, Ian Lyons and Robert I Richards, Howard Florey Institute, University of Melbourne, Parkville, 3052, Australia. The renin-angiotensin-aldosterone system plays an essential role in blood pressure and electrolyte homeostasis. In order to define the tissue-specific enhancers of the angiotensinogen gene in vivo, we constructed an angiotensinogen "minigene" by fusing the Bam HI sites from exons 2 and 4 of our mouse genomic clones. This internal deletion allows direct comparison of the endogenous and minigene mRNAs on a Northern blot using the same probe. Eight separate transgenic lines were studied. Minigenes containing 750 bp of promoter were expressed at high levels in the liver (9.9 and 2.4 fold the endogenous gene). In 4 lines containing 4 kb of 5'flanking region, hepatic expression was closer to that of the normal gene (1.5, 1.7, 2.9 and 1.7 times endogenous expression). The remaining 2 mouse lines did not express the construct. Tissue specificty directed by 4 kb of promoter was examined in one line (4.0 A/2: V). Parallel expression of the endogenous gene and the minigene was demonstrated in liver, brain, kidney, ovary and brown adipose tissue, whilst the submaxillary gland expressed the construct at excessive levels. We are currently using this model system to define the inducible enhancers of the angiotensinogen gene.

F 209 OVEREXPRESSION OF CHOLECYSTOKININ (CCK) IN TRANSGENIC MICE, J.M.Friedman[+], E.P. Sandgren[*], B.S.Schneider[++], R.D.Palmiter[+*], R.L.Brinster[*], [+]Howard Hughes Med.Inst., Rockefeller Univ.,NY,NY 10021, [*]School of Vet. Med., Univ. of Penn.,Philadelphia,PA 19104, [++]Long Island Jewish Hosp.,New Hyde Park,NY 11042, [+*]Howard Hughes Med. Inst.,Univ. of Wash., Seattle,WA 98195. CCK is a neuropeptide which has been characterized because of its ability to stimulate gallbladder contraction, pancreatic secretion and pancreatic growth. High levels of this peptide have also been found in brain where it is thought to function as a neurotransmitter. Studies of CCK in rodents with this peptide as well as CCK antagonists have also suggested that CCK may suppress appetite in vivo. Biologically active CCK is produced by a series of posttranslational modifications including proteolytic cleavage, amidation and sulfation of tyrosine. In order to consider the tissue distribution of the CCK processing machinery as well as the impact of overproduction of this peptide on gastrointestinal function and feeding behavior, we have placed the mouse CCK coding sequence under the control of the metallothionine promoter in transgenic mice. Posttranslationally processed and unprocessed cholecystokinin were distinguished by use of two separate antibodies which are specific for either the CCK precursor or the processed peptide. The transgenic animals have a 2-3 fold increase in the plasma levels of the processed hormone and a 1000-fold elevation in the levels of prohormone. High levels of precursor were found in multiple tissues but posttranslational cleavage and amidation of the transgene was apparent only in pituitary. These data suggest that the posttranslational processing machinery for this peptide is restricted and perhaps present only in neural cells. These animals are available for studies that will analyze the impact of CCK overexpression on GI physiology and feeding behavior.

F 210 POSITION INDEPENDENT HIGH LEVEL EXPRESSION IN TRANSGENIC MICE, Frank Grosveld, David Greaves, Dimitris Kioussis, Greet Blom van Assendelft, Phil Collis, Dale Talbot, Mike Antoniou and Georgina Lang. Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. U.K.

We have constructed a "mini" locus that contains the 5' and 3' flanking regions of the human beta-globin locus and the beta-globin gene. These regions are characterized by erythroid specific DNaseI superhypersensitive sites and are normally localized approximately 50kb 5' and 20kb 3' from the beta-globin gene. This "mini" locus is expressed tissue-specifically in transgenic mice at a level directly related to its copy number, independent of its position of integration in the mouse genome. Moreover, the expression per gene copy is the same in each mouse and as high as that of the endogenous mouse beta-globin gene. These results indicate that the DNA regions flanking the human beta-globin locus contain dominant regulatory sequences that specify position independent expression and normally activate the complete human multigene beta-globin locus. We have now mapped these control sequences individually and have shown that they can act on heterologous genes. We have identified similar sequences in the T-cell specific gene CD2. These also act on heterologous genes to allow position independent expression in T-cells of transgenic mice. Further characterization of these sequences will be discussed.

F 211 TECHNIQUES FOR THE DEVELOPMENT OF TRANSGENIC FISH : A REVIEW, Daniel Chourrout[1], René Guyomard[1] and Louis-Marie Houdebine[2], Laboratory of fish genetics[1], Unit of cell and molecular differentiation[2], National Institute for Research in Agriculture, 78350 Jouy-en-Josas, France.
Transgenic fish are of interest for biological studies and for genetic improvement in aquaculture. Advantages of fish over mammals are a usually high fertility and an external fertilization which permit the manipulation of numerous embryos. However, egg pronuclei have not yet been visualized and fish retroviral vectors are not available. The present review describes the production of numerous transgenic fish by injections of various gene constructions into the egg cytoplasm. Injection into the oocyte germinal vesicle is another possibility. Germ-line transmission has been demonstrated in the rainbow trout and the zebrafish. Preliminary observations of foreign gene expression in transgenic fish and in transfected fish cells are also presented.

F 212 RETROVIRAL GENE INSERTION AND EXPRESSION IN MOSAIC CHICKENS, *Marcos D. Hartitz, ^Mary M. McGrane, *Renee Z. Fisk, and *Clague P. Hodgson, Edison Animal Biotechnology Center, *Labs of Molecular and Developmental Biology/Dairy Science, Ohio Agricultural Research and Development Center/The Ohio State University, Wooster, OH. 44691, ^Dept. of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH. 44106.
A replication competent avian sarcoma virus derived vector was used to deliver a foreign gene cassette [the rat phosphoenolpyruvate carboxykinase (PEPCK) gene promoter driving expression of bacterial neomycin resistance (neo) structural gene sequences] into quail qt6 cells and SPAFAS line 11 chicken embryos. Cell lines (resistant to the drug G418) resulting from infection or transfection with the construct contained the foreign genes as well as frequently rearranged viral genomes. Chickens hatched from eggs infected with the recombinant retrovirus at day zero of development contained chromosomally integrated viral genomes bearing the foreign genes. Tissues of infected chickens displayed a mosaic pattern of insertion in the fully developed adult animal, with the vectored genes appearing in some tissues but not others in an unpredictable fashion. In particular, blood tended to become negative for the foreign genes as animals aged while other tissues, such as heart muscle, remained positive. Expression of either PEPCK-neo mRNA, or intact viral RNAs was detected in cell lines and tissues of mosaic animals.

F 213 GENERATION OF TRANSGENIC SHEEP BY SUB-ZONAL INJECTION OF FELINE LEUKAEMIA VIRUS, Simon J.H. Hettle[1], Michael J.A. Harvey[2], Ewan R. Cameron[2], Carolyn S. Johnston[1] and David E. Onions[1]. Departments of Veterinary [1]Pathology and [2]Reproduction, University of Glasgow Veterinary School, Glasgow G61 1QH, Scotland, U.K. Pronuclear microinjection of naked DNA fragments is the most common current method of production of transgenic mice as it is both relatively simple and successful(1). This technique is much more difficult to apply to domestic livestock, however, due to technical difficulties, especially problems of pronuclear visualisation(2). Retroviral vectors have also been used in producing transgenic mice(3) and we have now used a feline leukaemia virus (FeLV) to generate transgenic sheep. A concentrated virus preparation (c.5 x 10^6 ffu/ml) was injected into the perivitelline space of 2-4 cell ovine embryos and the embryos replaced in the oviducts of foster ewes. These ewes were slaughtered at 50 days' gestation, the embryos recovered and their DNA analysed by Southern hybridisation. FeLV specific sequences were detected in the genomes of 2/17 (11.76%) embryos. Embryo survival in both injected and non-injected embryos was c.50%. We hope to develop an FeLV-based packaging cell line and vector system for use in the sheep akin to those used in mice(4).

1) Brinster et al. (1985) Proc.Nat.Acad.Sci. (USA) 82, 4438-4442.
2) Clark et al. (1987) Trends Biotechnology 5, 20-24.
3) van der Putten et al. (1985) Proc.Nat.Acad.Sci. (USA) 82, 6148-6152.
4) Miller & Buttimore (1986) Mol.Cell Biol. 6, 2895-2902.

F 214 DOMINANT NEGATIVE MUTATION IN TCRβ CHAIN GENE CAUSES T CELL DEPLETION IN TRANSGENIC MICE, Paul Krimpenfort and Anton Berns, Div. of Molecular Genetics of the Netherlands Cancer Institute and Dep. of Biochemistry, University of Amsterdam, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
In transgenic mice the introduced functional TCRβ gene prevents expression of endogenous β genes by inhibiting complete VDJ joining. To study the molecular requirements for this phenomenon in more detail, we have constructed a mutant TCRβ chain gene (ΔV-TCRβ), which by analogy to the Ig Dμ-chain encodes a β chain polypeptide lacking the variable region. In transgenic mice carrying the mutant TCRβ gene no or only remnants of thymus tissue was found. FACS analysis using antisera specific for B and T cell surface markers showed T cell depletion in the spleen of the ΔV-TCRβ transgenic mice. We suggest that the mutant TCR polypeptide inhibits the rearrangements of endogenous β genes and thereby prevents the membrane expression of functional heterodimeric TCRs, leading to the abrogation of normal T cell maturation. We are crossing the ΔV-TCRβ transgenic mice with transgenic mice carrying a functional TCRβ chain gene: expression of a functional gene might rescue the mutant phenotype and thereby provide insight into the mechanism of T cell depletion in ΔV-TCRβ transgenic mice.

F 215 METABOLIC ALTERATIONS CAUSED BY THE EXPRESSION OF bGH IN TRANSGENIC ANIMALS, M.M. McGrane, J. Yun, G. Hendrick, T. Wagner, B. Arafah, G. Weir and R.W. Hanson, Edison Animal Biotechnology Center, Case Western Reserve University, Cleveland, OH 44106 and Ohio University, Athens, OH 45701 and Diabetes Center, Joslin Found., Boston, MA 02215. Alterations in carbohydrate metabolism in transgenic mice containing the PEPCK/bGH and MT/bGH genes were measured and correlated with the developmental expression of the two transgenes. PEPCK/bGH was not expressed until 19 days of fetal life, whereas MT/bGH mRNA was detected at high levels early in development. Adult MT/bGH mice were hyperinsulinemic (insulin = 8.0 ng/ml vs. 0.6 ng/ml) and relatively hypoglycemic (5.5 mM vs 4.6 mM) when compared to controls. PEPCK/bGH animals had insulin levels of 0.5 ng/ml and glucose levels of 4.6 mM. The concentration of insulin-like growth factor-1 (IGF-1) in PEPCK/bGH mice was 1.5 μg/ml which was double that found in control animals. These animals were also accutely sensitive to pharmacologic doses of insulin, although the glucose concentration was reduced to the same level as control animals. As a result of the elevated concentration of IGF-1 in PEPCK/bGH mice, the hepatic expression of the genes for PEPCK and tyrosine aminotransferase was reduced to 10% of that in normal animals. We conclude that the metabolic effects of bGH in transgenic mice are dependent in part on the developmental and tissue-specific expression of the transgene as well as the concentration of bGH in the blood of animals. Supported by Grants DK 21859, DK 24451 and funds from the Edison Program of the State of Ohio.

F 216 EXPRESSION OF THE HUMAN PROTEOLIPID PROTEIN GENE IN TRANSGENIC MICE. N. Nadon, H. Arnheiter, S. Chang and L. Hudson. NIH, NINCDS, Bethesda MD 20892.
Proteolipid protein (PLP) is the most abundant protein in central nervous system (CNS) myelin. This protein is highly conserved between species and is thought to play a role in the compaction of the myelin sheath. Loss of PLP function in the X-linked jimpy mouse mutation results in severe tremors, a dearth of mature oligodendrocytes, and hypomyelination of the CNS, with death occurring by 3-4 weeks. The jimpy phenotype appears to be the result of a point mutation in the PLP gene that results in incorrect splicing of the PLP mRNA. We have produced transgenic mice carrying a human PLP gene construct to analyze the regulation of the PLP gene and to demonstrate that restoration of the normal PLP protein to jimpy mice results in the moderation or abolition of the pleiotropic affect of the jimpy mutation. A construct was made containing the human PLP cDNA with about 4.5 kb of 5' flanking genomic DNA and 1.5 kb of 3' flanking genomic DNA from the PLP gene. This construct contains no introns or splice junctions. Three founder lines of transgenic mice were obtained with this construct, which resulted in four lines of transmission, as the DNA integrated at two sites in one founder. Three of the lines expressed the transgene mRNA at very low levels (<2% endogenous PLP levels). The fourth line, carrying >30 copies of the transgene, expressed the mRNA at near endogenous PLP levels. In all cases the expression was tissue-specific, with transgene mRNA found in the brain, but not in kidney, liver, lung, spleen or heart. These results demonstrate that all the regulatory sequences necessary for controlling the high level of tissue-specific PLP expression are contained within the construct injected. The high-level expression line has been bred to jimpy carriers, and male offspring carrying the jimpy mutation and the transgene will be analyzed to determine if the transgene mRNA results in protein that gets incorporated into myelin and if the presence of this transgene derived protein "cures" the jimpy phenotype.

F 217 TRANSIENT EXPRESSION OF THE BACTERIAL CAT GENE DIRECTED BY HETEROLOGOUS PROMOTERS DURING EARLY XENOPUS DEVELOPMENT. Per-Eric Olsson, Mohammad Zafarullah, Randy Foster, Leon W. Browder and Lashitew Gedamu. Department of Biological Sciences, University of Calgary, Alta, Canada T2N 1N4. Functionality and developmental control of heterologous metallothionein (MT) promoters were investigated in early Xenopus embryos. One-cell stage embryos were microinjected with 5 pg of supercoiled plasmid DNA in which the bacterial CAT reporter gene was under the transcriptional control of three different MT promoters. These were the 5′ flanking fragments of the human MTII-A (800 bp), the human MTI-G (630 bp) and the rainbow trout MT-B (720 bp) genes. The microinjected embryos were analysed for CAT activity at 6, 12, 24, 48 and 72 hours postfertilization. CAT activity was detected in the 12, 24 and 48 hour embryos with maximum activity at 24 hours. This study was supported by AHFMR, MRC and NSERC of Canada.

F 218 GENE TRANSFER TO SWINE EMBRYOS USING AN AVIAN RETROVIRUS, Robert M. Petters, Bryan H. Johnson and Ruth M. Shuman, Departments of Animal Science and Poultry Science, North Carolina State University, Raleigh, NC 27695. Blastocyst-stage swine embryos were microinjected with cultured dog cells producing an avian retrovirus, spleen necrosis virus (SNV). Between 100 and 150 cells were injected into the blastocoel of each embryo. Two hundred and twenty-two injected embryos were transferred to 12 recipient female pigs. From 4 pregnant recipient females, 21 normal fetuses were recovered at 6 wk of gestation. DNA was extracted from fetal tissues including brain, heart, liver, and thigh muscle. We applied a polymerase chain reaction (PCR) procedure to amplify a SNV target sequence. If SNV sequences were present in the DNA samples from the fetuses, the target sequence would be amplified and detectable in slot-blot hybridizations. Seventeen of twenty-one fetuses had at least one tissue that contained the SNV sequence as determined by slot-blot hybridization. Detection of SNV sequences in DNA samples from 6 wk fetuses derived from embryos exposed to SNV indicates that SNV can infect early swine embryos and become incorporated into the swine genome. This heterologous gene transfer system (avian retrovirus in mammals) holds much promise for practical application in livestock species such as swine.

F 219 TISSUE-SPECIFIC AND HORMONE-REGULATED EXPRESSION OF MILK PROTEIN GENES
Christoph W. Pittius, Henryk Lubon and Lothar Hennighausen, LBM, NIDDK, National Institutes of Health, Bethesda, Maryland 20892
Control elements governing milk protein gene expression have been studied *in vitro*, in tissue culture cells and in transgenic animals. The promoter/upstream region of the whey acidic protein (WAP) gene confers mammary-specific expression of a marker gene in transgenic animals (Pittius et al., Proc. Natl. Acad. Sci. USA 85: 5874-5878 (1988)) and contains some, but not all control elements conferring hormone regulation (Pittius et al., Mol. Endocrinology, in press). *In vitro* studies have shown that nuclear proteins specific to the mammary gland and general transcription factors bind to the promoter/upstream region of the WAP gene (Lubon and Hennighausen, Nucl. Acids Res. 15: 2103-2121 (1987)). Using *in vitro* transcription and gene transfer assays we can show that WAP gene sequences between -355 and -25 activate transcription in mammary and non-mammary cells and contain glucocorticoid-responsive elements. The WAP gene promoter itself is not transcribed in non-mammary cell lines, suggesting that it contains regulatory elements responding only or preferentially to the transcriptional machinery in the lactating mammary gland. We are currently in the process of using these findings to construct an expression vector for tissue-apecific and correctly hormone-regulated expression of foreign proteins in the mammary gland of transgenic dairy animals.

F 220 FISH ANTIFREEZE PROTEIN GENES UNDER YP 1,2 TRANSCRIPTIONAL CONTROL IN Drosophila. Derrick E. Rancourt, I.D. Peters, T.M. MacGregor, V.K. Walker*, and P.L. Davies. Depts. of Biochemistry and Biology*, Queen's University, Kingston, CANADA K7L 3N6.

Antifreeze proteins (AFP) are serum proteins produced by specific groups of freeze-resistant marine fish and terrestrial insects which depress body fluid freezing points. In marine fish, AFP at 10 mg/ml can lower the freezing point of serum by ≈1C° thereby enabling these fish to survive in the coldest of sea water temperatures.

We are investigating whether freeze resistance can be conferred to other organisms through gene transfer using Drosophila as a model system. We have previously reported that Hsp70-AFP transcripts are properly processed and translated in Drosophila. However, to detect biological activity it is necessary to increase the levels of AFP gene expression. We have placed two wolffish (Anarhichas lupus) AFP genes under the divergent transcriptional control of the Drosophila Yolk Protein (YP)1,2 intergenic region by fusing genes within the signal peptide coding regions. Since levels of YP reach 50 mg/ml in female hemolymph, promoters from these genes are good candidates for increasing AFP levels. Transformants containing these genes show processed AFP transcripts in vitellogenic females which are translated and yield significant levels of biologically active AFP in hemolymph. Supported by MRC and NSERC of Canada.

F 221 CIS-ACTING ELEMENTS REQUIRED FOR THE TISSUE-SPECIFIC EXPRESSION OF THE RAT β-CASEIN GENE IN TRANSGENIC MICE, Theresia V. Reding, Norman M. Greenberg, Kuo-Fen Lee and Jeffrey M. Rosen, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030-3498

Previous studies from our laboratory have demonstrated the tissue- and stage-specific expression of the entire rat β-casein gene (Nucl.Acids Res. 16:1027-1041, 1988), and casein promoter-bacterial chloramphenicol acetyltransferase (CAT) fusion genes (Mol. Cell Biol., in press) in the mammary gland of transgenic mice. A -524/+490 casein promoter fragment containing both 5' flanking, exon I and intron A sequences was sufficient to elicit tissue- and stage-specific expression of CAT. In order to further define the cis-acting sequences required for these specificities we have generated lines of mice containing a -524/-13 promoter-fusion lacking the highly conserved exon I and the less well conserved intron A sequences. In addition, mice carrying a putative mammary consensus sequence, linked to a heterologous promoter have been produced. The consensus was identified by sequence comparisons between a number of different milk protein genes. Gel-shift and footprinting experiments using nuclear extracts from mammary and non-mammary cell lines with a conserved sequence oligonucleotide have demonstrated specific protein-DNA interactions. The effects of a heterologous enhancer on the level and specificity of expression of the casein-CAT fusion genes have also been analyzed. These studies should facilitate vector design for targeting the efficient synthesis and secretion of foreign proteins into the milk of transgenic livestock. (Supported by USDA grant 86-CRCR-1-2250 and NIH grant CA16303.)

F 222 TOWARDS A NEW SHEEP GENOTYPE WITH INCREASED WOOL GROWTH BY TRANSGENESIS WITH MICROBIAL GENES FOR CYSTEINE SYNTHESIS. George E. Rogers, A.V. Sivaprasad, Richard J. D'Andrea, C. Simon Bawden and Elizabeth S. Kuczek, Department of Biochemistry and Centre for Gene Technology, University of Adelaide, South Australia, Australia 5000.

Through recombinant-DNA technology it should be possible to confer on sheep the capability of synthesizing essential amino acids. There is abundant evidence that normal wool growth is sub-optimal because of a net loss of cysteine-sulphur during microbial protein metabolism in the rumen and that cysteine (or methionine) supplementation increases wool growth. Thus our present objective is to establish endogenous cysteine synthesis in the ruminal mucosa by transgenising sheep with genes for the two enzymes essential for cysteine synthesis. These are serine acetyltransferase (SAT) and O-acetylserine sulphydrylase (OASS) either from *Salmonella typhimurium* (*cysE* and *cysM*, respectively) or from *Saccharomyces cerevisiae* (*MET25* for OASS; the *cysE* equivalent gene in yeast was putatively *CYS1*, but we have found that it encodes β-cystathionase). The genes *cysE*, *cysM* and *MET25* have been isolated, sequenced and fused to selected eukaryotic promoters and 5′ upstream sequences; one source of such sequences being investigated is the 5′ region of keratin genes which are specifically expressed in the ruminal mucosa, a keratinizing epithelium. Our experiments have led to the expression of the genes to give active enzymes (1) in the SP6/T7 transcription/reticulocyte lysate translation system, (2) in stably transfected animal cells (CHO) in culture, and (3) in transgenic mice. Detection of the enzyme activities in the above three expression systems has been facilitated by a sensitive and simple T.L.C. assay procedure. Apart from the commercial aspect, the successful introduction of an essential amino acid biosynthetic pathway into a mammal may provide exciting avenues for fundamental studies in other areas of animal biology.

F 223 DIFFERENTIAL EXPRESSION OF THE ACTIN GENE FAMILY IN TRANSGENIC MODELS, Arthur T. Sands, Franco DeMayo, Robert Schwartz, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030. Portions of the 5' regulatory sequences of muscle-specific actin genes have been fused to a bacterial reporter gene, chloramphenicol acetyl transferase (CAT), and inserted into transgenic mice. 411 base pairs of the α-skeletal actin promoter and 318 base pairs of the α-cardiac promoter have produced CAT expression with high degrees of specificity for cardiac and skeletal muscle. In addition, portions of the 5' regulatory sequences of muscle-specific and non-muscle type actin genes have been inserted into a retroviral vector where they are linked to a reporter gene that codes for the enzyme β-galactosidase. The constructs are based on a non-replicative, avian spleen necrosis viral vector developed by Howard Temin. The promoter regions utilized in this study are derived from the 5' regulatory regions of the α-skeletal, α-cardiac, α-smooth, and β-cytoplasmic actin genes. Enzymatic activity will be assayed as a marker for promoter activity after infection of primary cell cultures and subsequently avian embryos. Previous transfection studies of primary cell cultures have shown these promoter regions to confer appropriate, tissue-restricted expression of the CAT gene.

F 224 The Effects of Eukaryotic Methylation on Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice.
Jay M. Short, Melody Blakeley, Joseph A. Sorge, William D. Huse, & Steven W. Kohler, Stratagene, 11099 N. Torrey Pines Rd., La Jolla, CA 92037.

Transgenic mice, which contain intact copies of a bacteriophage lambda shuttle vector, have been generated for the development of a short-term mutagenicity assay. Using *in vitro* lambda packaging extracts, the shuttle vector has been successfully rescued from genomic DNA isolated from a variety of mouse tissues. Mutations in a beta-galactosidase target gene positioned within the shuttle vector are identified by color assay on indicator plates. The effects of eukaryotic methylation on lambda phage rescue were investigated using 5-azacytidine treated fibroblast cultures derived from the transgenic mice. The increased efficiency of rescue following 5-azacytidine treatment correlated with a decrease in methylation as measured by restriction digestion with methylation sensitive enzymes. Alternative approaches that overcome the effects of methylation in the animals without the use of 5-azacytidine will be discussed. The use of lambda shuttle vectors in transgenic animals will permit the development of a reliable and sensitive short-term *in vivo* assay for assessing the mutagenicity of toxic substances.

F 225 **REGULATION OF MOUSE MAJOR URINARY PROTEIN (MUP) GENE EXPRESSION,** **H. Jin Son[1], K. Shahan[2], Y. Shi[2], E. Derman[2] and F. Costantini[1], Dept. of Genetics and Development, Columbia University, New York, NY 10032[1] and The Public Health Research Institute, New York, NY 10016[2].** Mouse major urinary proteins (MUPs) are low M.W. secreted proteins of unknown function, believed to bind a small lipophilic ligand. The MUP gene family is of particular interest because while its members are extremely homologous, they are subject to widely divergent patterns of tissue-specific, developmental and hormonal control. We have investigated a pair of nearly identical MUP genes, encoding the "MUP V" type of mRNA found predominantly in the submaxillary gland. Transgenic mice carrying the MUP V_β gene with 5 kb of 5' flanking DNA expressed the gene specifically in the submaxillary gland at 3-5 times the endogenous level. In contrast, the same gene with 0.5 kb of 5' DNA was expressed at 20-100 fold lower levels, and in a non-tissue-specific pattern. These results begin to localize the cis-acting regulatory elements required for tissue-specific expression, and experiments to further delineate these sequences are in progress. Surprisingly, recent evidence has shown that the endogenous MUP V_β gene is normally silent, and only the MUP V_α gene is expressed in the submaxillary gland. Nevertheless, the MUP V_β gene remains capable of efficient, tissue-specific expression when excised from its normal genomic locus and used as a transgene. Possible models to explain this apparent discrepancy will be discussed.

F 226 TRANSGENIC MICE TO STUDY THE ROLE OF THYMIC EPITHELIUM

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Most of the critical steps in the differentiation of T-cells occur in the thymus. However, the precise molecular mechanisms underlying the proliferation, differentiation, and maturation of thymocytes are poorly understood. Apart from soluble mediators such as interleukins, it is dependant on complex cell-cell interactions with stromal cells which mainly consist of macrophages/dendritic cells and a heterogeneous epithelium. The development of athymic systems to study T-cell differentiation may mainly be hampered because of the complex interplay and tight cell-cell interactions in vivo. Hence, in vivo systems to study the role of the thymic microenvironment seem more promising. We have generated transgenic mice that specifically express the SV40 T-antigen oncogene in a subclass of thymic epithelial cells. These mice develop gross thymic hyperplasia which involves both epithelial cells and thymocytes. To determine its cause we analyzed expression of lymphokine genes, thymocyte development (FACS), and epithelial cell differentiation. A model will be discussed to explain the hyperplastic phenotype and the role of the epithelial cell type in thymocyte differentiation in vivo.

F 227 **EXPRESSION OF THE HUMAN β-AMYLOID PRECURSOR GENE IN TRANSGENIC MICE.**

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We are investigating the relationship between the amyloid precursor protein (APP) and the formation of amyloid in Alzheimer's disease. Experiments will be presented to study the developmental regulation and tissue-specific expression of the APP gene in vivo, and to assess the relative levels of alternative transcripts derived from the APP gene in normal and pathological brain tissue.

Various gene constructs containing the APP regulatory region and APP gene transcripts have been introduced into mice. These transgenic mice provide a unique opportunity for studying the differential expression pattern of the APP gene, and for studying the biological effects of this gene in vivo. Furthermore, these animals can be used to generate models of disease, which will be discussed.