MOLECULAR BIOLOGY OF HUMAN GENETIC DISEASE

Organizers: Maimon M. Cohen, Beverly S. Emanuel,
David H. Ledbetter and Arthur L. Beaudet
April 3-10, 1992

Plenary Sessions Page

Poster Sessions

Mutational Analysis

U 001 HEMOPHILIA A AND A HUMAN TRANSPOSABLE ELEMENT, Haig H. Kazazian, Jr., Beth A. Dombroski, Miyoko Higuchi, Alan F. Scott, Steve Mathias, Abram Gabriel, Jef D. Boeke and Stylianos E. Antonarakis. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Because it is important to give accurate counselling in clinical situations, a major problem in human genetics involves the need to characterize rapidly mutant alleles in large genes. In many autosomal dominant and X-linked disorders, nearly all unrelated affected individuals will carry different mutant alleles. We set out to find the mutant alleles in one such disorder, hemophilia A, an X-linked condition. In early analyses, we detected only 10% of mutant alleles by Southern blotting, but we learned about mutation hotspots and transposable elements in human (see below). Recently we used denaturing gradient gel electrophoresis to screen 78 individuals for point mutations in the factor VIII gene which is defective in hemophilia A. After computer analysis, the 8kb of protein coding and intervening sequences were assayed as 47 distinct PCR fragments. Mutations were found in 75% of total patients, but in only 55% of patients with severe hemophilia A. While this method is a good screening method, it seems that it is necessary to look outside of the coding region of the gene for a proportion of mutations in severe hemophilia A. Whether this problem will be faced in other genetic diseases remains to be determined.

During this search, we found two de novo insertions of truncated L1 elements into the factor VIII gene on the X chromosome that produced hemophilia A. We have identified a full length L1 element that is the likely progenitor of one of these insertions by its sequence identity to the factor VIII insertion. This L1 element contains two open reading frames, both of which are expressed in experimental systems. The second ORF encodes reverse transcriptase activity. This L1 element is one of at least four alleles of a locus on chromosome 22 that has been occupied by an L1 element for at least 6 million years.

Cancer Genetics (Joint)

U 002 **REGULATING A "MASTER REGULATOR": CDK CONTROL OF THE RETINOBLASTOMA PROTEIN.**

Philip W. Hinds[1], Sibylle Mittnacht[1], Steven F. Dowdy[1], Andrew Arnold[2], Steven I. Reed[3], and Robert A. Weinberg[1], [1]Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, [2]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, and [3]The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The retinoblastoma tumor suppressor protein (pRb) is thought to regulate passage through the cell cycle. In normal cells, pRb itself may be negatively regulated by phosphorylation, such that only the hypophosphorylated form of pRb is functional in blocking cell cycle progression. In some tumor cells this block is alleviated by mutational inactivation of pRb. Consistent with this idea, restoration of pRb expression in a variety of tumor cell lines leads to a loss of proliferative or tumorigenic capacity. In the human osteosarcoma cell line SAOS-2, this suppression of proliferation is accompanied by a marked phenotypic change, in that cells transiently transfected with pRb-encoding constructs become greatly enlarged. When co-transfected with a drug-resistance marker, these "flat cells" persist in culture without division for weeks. Tumor-derived and some in vitro pRb mutants are unable to elicit this phenotype, suggesting that the production of flat cells is a consequence of the tumor suppressor function of pRb. Wild-type pRb expressed in SAOS-2 cells is predominantly hypophosphorylated and associated with nuclear structures, consistent with the idea that this form of pRb is functional in suppression of proliferation. Indeed, the apparently total lack of phosphorylation of pRb upon transient expression in SAOS-2 cells suggests that the susceptibility of these cells to pRb may be due to levels of pRb that overwhelm the endogenous kinase system normally responsible for cyclically inactivating pRb. As many of the phosphate residues on pRb are thought to be added by a member(s) of the cdk family of kinases, the regulatory subunits of these kinases, known as cyclins, have been cotransfected with pRb into SAOS-2 cells in an attempt to provide the inactivating mechanism which is apparently normally insufficient in these cells. Two human cyclins, cyclin E and cyclin A, lead to a nearly complete loss of flat cell phenotype, and the pRb produced in the transiently transfected cells failed to associate with the nucleus due to hyperphosphorylation. In addition, two pRb mutants were identified which are resistant to this phenotypic "rescue" mediated by the cyclins, suggesting that cyclin overexpression directly inactivates pRb, rather than circumventing it. These results present evidence that the cyclin/cdk system is responsible for controlling the functional state of pRb, and thus the capacity for cell cycle progression.

U 003 TUMOR SUPPRESSOR GENES AND HEREDITARY TUMOR SYNDROMES IN THE HUMAN NERVOUS SYSTEM: TOWARD THE ISOLATION OF THE GENE CAUSING NEUROFIBROMATOSIS TYPE 2 (NF2), Bernd R. Seizinger[1],[2], Nikolai Kley[1], Anil Menon[2], Terry Lerner[2], Mia MacCollin[2], James Trofatter[2], Jonathan Haines[2], James Gusella[2], Ronald Lekanne-Deprez[3], Ellen Zwarthoff[3],

[1]Molecular Neuro-Oncology Laboratory, [2]Molecular Neurogenetics Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, U.S.A., [3]Department of Pathology, Erasmus University, Rotterdam, The Netherlands.

Many human cancers are known to occur in two different forms: as sporadic tumors in the general population, and as hereditary tumors within families. Hereditary tumor syndromes offer unique model systems for isolating genes whose mutations lead to cancer. There is accumulating evidence that hereditary and sporadic tumors are caused by similar pathogenetic mechanisms affecting the same genes. The cloning and characterization of these genes may therefore have important implications for diagnosis and treatment not only of the relatively rare hereditary tumors, but also for their much more common sporadic counterparts.

Recombinant DNA technology has provided a powerful strategy for investigating inherited diseases in which the defective protein is unknown. This strategy has been termed "reversed genetics" because the first goal is not to find the defective protein, but rather to isolate the defective gene based on the determination of its chromosomal location in the human genome. However, most genes which have been cloned thus far using the "reversed genetics" approach, have been isolated with the additional aid of cytogenetically visible chromosomal aberrations, providing potential short-cuts to the defective genes. For example, the gene for neurofibromatosis type 1 (NF1) was recently identified by cloning the translocation breakpoints of two NF1 patients with constitutional translocations in the NF1 region on chromosome 17q11.2 (see first speaker).

NF is one of the most common inherited disorders affecting the human nervous system. There are two clinically and genetically distinct forms of NF: NF1 (von Recklinghausen NF) and a less frequent form, NF2 (bilateral acoustic NF). Although different cell types may be affected, the most common abnormalities in both NF forms are in the nervous system, in cells of neural crest origin. Acoustic neuromas, Schwann cell-derived tumors of the 8th cranial nerve, are the hallmark of NF2 and frequently lead to deafness. Furthermore, meningiomas and other neural-crest derived tumors such as spinal Schwannomas, are common in NF2 patients. We have previously shown that the gene causing NF2 maps to chromosome 22q. The region containing the NF2 gene is frequently deleted in tumors associated with NF2, in both their sporadic and hereditary forms, including acoustic neuromas, spinal Schwannomas, and meningiomas, suggesting that the NF2 gene belongs to the family of "tumor suppressor" genes, i.e., genes which normally confer growth suppression, and whose loss of function is associated with tumor formation.

The recent discovery of a meningioma tumor specimen with a translocation on chromosome 22 (Zwarthoff et al.) has provided a potential short-cut to the isolation of the NF2 gene. We have shown that the translocation breakpoint maps to the region on chromosome 22 identified as the NF2 region, based on tumor deletion and linkage studies. We have meanwhile identified flanking markers of the translocation breakpoint which recognize the translocation breakpoint on pulsed-field gel electrophoresis. These and other markers also appear to identify constitutional aberrations in patients with NF2 and multiple meningiomas. Thus, the search for transcripts in this region which are expressed in normal Schwann cells, but not in Schwann cell-derived tumors, may eventually lead to the identification of the NF2 gene and/or the or a meningioma "tumor suppressor" locus on chromosome 22.

Sex Differentiation Hormone Receptors

U 004 RETINOID RECEPTORS IN DEVELOPMENT AND DISEASE, Ronald M. Evans[1], Akira Kakizuka, Steve Kliewer David Mangelsdorf, and Kazuhiko Umesono[1], Howard Hughes Medical Institute[1], The Salk Institute, La Jolla, CA 92037.

The cellular responses to RA are mediated by two families of transcription factors, which include the RA receptors (RARs) and the retinoid X receptors (RXRs). Although both RAR and RXR respond specifically to RA, they differe substantially from one another in primary structure and ligand specificity. A major question raised by the discovery of two retinoid-responsive systems is whether their functions are independent, interactive, or redundant. One approach to answer this question is to determine whether they share common or distinct downstream target genes. In regard to target sequences we have recently described properties of direct repeats (DRs) of the half-site AGGTCA as hormone response elements. According to our results, spacing of the half-site by 3, 4, or 5 nucleotides determines specificty of response for vitamin D3, thyroid hormone and retinoic acid receptors, respectively. This so-called "3-4-5" rule suggests a simple physiologic code exists in which half-site spacing plays a critical role in achieving selective hormonal response. As part of these studies, we have also identified that the RXR, but not the RAR, is able to activate through a direct repeat spaced by one nucleotide. In contrast, both RAR and RXR are able to commonly activate through a DR with a spacing of 5. Evidence that RXR heterodimers modulate the RA response will be presented.

Finally we will discuss the isolation and characterization of a fusion product produced as a consequence of a t(15;17) translocation characteristic of human acute promyelocytic leukemia. This translocation which occurs in the retinoic acid receptor gene generates a unique mRNA which encodes a fusion protein between the retinoic acid receptor alpha (RARα) and a myeloid gene product called PML. Structural analysis reveals that the PML protein is a member of newly recognized protein family that includes a variety of putative transcription factors as well as the recombination-activating gene product (RAG-1). The abberant PML-RAR fusion product, while typically retinoic acid responsive, displays both cell type and promoter specific differences from the wild type RARα. Because patients with APL be induced into remission with high dose RA therapy, we propose that the non-liganded PML-RAR is a new class of dominant negative oncogene product.

Microdeletion Syndromes

U 005 MICRODISSECTION AND MOLECULAR ANALYSIS OF CHROMOSOME REGIONS INVOLVED IN SEGMENTAL ANEUSOMY Bernhard Horsthemke, Institut für Humangenetik, Universitätsklinikum Essen, Germany

Using microdissection and enzymatic DNA amplification, we have recently isolated DNA markers for the Langer-Giedion syndrome chromosome region (LGCR, 8q24.1), the Prader-Willi/Angelman syndrome chromosome region (PWCR/ANCR, 15q11-13) and other chromosome regions. The microclones are being used to define the critical regions at the molecular level, to construct STS-nucleated YAC contigs and to identify gene sequences. Microclone L48, which was isolated from the LGCR library, defines the shortest region of deletion overlap in Langer-Giedion syndrome. Microclone MN7, which was isolated from the PWCR/ANCR library, detects four or five closely related gene loci in 15q11-13 and one or two related gene loci in 16p11.2. Transcripts of 14 and 8 kb are present in placenta, brain and other human tissues. The presence of several closely related DNA sequences in proximal 15q may conceivably contribute to the genetic and physical instability of this chromosome region and thus to the etiology of associated disorders. (Supported by the Deutsche Forschungsgemeinschaft)

U 006 GENETIC IMPRINTING AND THE ANGELMAN/PRADER-WILLI SYNDROMES : EVIDENCE THAT THE DISEASE LOCI ARE NOT IDENTICAL. Marc Lalande[1,2], Joan H.M. Knoll[1], Daniel Sinnett[1], Shinji Saitoh[3], Norio Niikawa[3] and Joseph Wagstaff[1], [1]Genetics Division, Children's Hospital and Department of Pediatrics, Harvard Medical School, 300 Longwood Ave., Boston, MA 02115, [2]Howard Hughes Medical Institute and [3]Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki 852, Japan

The majority of patients suffering from the distinct genetic disorders, Prader-Willi syndrome (PWS) and Angelman syndrome (AS), display a deletion of the chromosome 15q11q13 region which is similar in size when analyzed both cytogenetically and molecularly. There are, however, several genetic differences between AS and PWS. In deletion cases, the parental origin of the 15q- is exclusively paternal in PWS but always maternal in AS. In nondeletion cases of PWS, there is also absence of a paternal contribution to 15q11q13 due to the occurrence of uniparental maternal disomy in most instances. These findings are consistent with the occurrence of genetic imprinting in the AS/PWS region. Uniparental paternal disomy of 15q has been detected in a few cases of AS although the incidence of uniparental disomy appears to be rare in nondeletion AS. The involvement of dissimilar molecular mechanisms in most nondeletion cases of AS and PWS suggests that a different gene or set of genes could be associated with PWS and AS. In support of this hypothesis, we have recently detected a difference in the distal extents of the AS and PWS critical regions of deletion overlap and mapped a neurotransmitter receptor gene to the region where this difference is observed. In addition, a submicroscopic deletion encompassing this $GABA_A$ (gamma-aminobutyric acid) receptor β3 subunit gene is detected in three AS siblings as well as their phenotypically normal mother and maternal grandfather. The latter results provide strong evidence that the AS and PWS genetic loci are distinct since paternal transmission of the submicroscopic deletion in this family does not produce an abnormal phenotype, whereas maternal transmission results in AS. If the same genes or set of genes were involved in the two disorders, paternal transmission would have resulted in PWS. It would appear, therefore, that linked, but not identical, loci with different patterns of imprinting exist or AS and PWS.

U 007 MOLECULAR ANALYSIS OF THE MILLER-DIEKER LISSENCEPHALY REGION (17P13.3), David H. Ledbetter[1], Susan A. Ledbetter[1], Akira Kuwano[1], Romeo Carrozzo[1] and William B. Dobyns[2], [1]Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, [2]Departments of Neurology and Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46202.

Lissencephaly (agyria, pachygyria) is a severe brain malformation manifested by a smooth cerebral surface, resulting from an arrest of neuronal migration at 10-14 wk gestation. Type I, or classical, lissencephaly can occur either in association with Miller-Dieker syndrome (MDS) or as an isolated finding, termed "isolated lissencephaly sequence"(ILS). Approximately 90% of MDS patients (34/37) have cytogenetically visible or submicroscopic deletions of 17p13.3. Recently, we have shown that 20% of ILS patients (10/51) have submicroscopic deletions within the same region. The centromeric boundaries of MDS and ILS deletions are quite variable and overlap each other, while the ILS deletions do not extend as far in the telomeric direction as do most MDS deletions. Pulsed-field gel analysis and yeast artificial chromosome (YAC) cloning have established the size of the critical region as less than 350 kb. Using the St. Louis and CEPH total human YAC libraries, we have established a complete YAC contig of this interval. A NotI linking clone (LL132) mapping within this interval shows cross-hybridization to rodent DNA and has been used to screen two human fetal brain cDNA libraries (Stratagene, Clontech). A contig of 3.5 kb of cDNA, containing a canonical polyadenylation signal, has been assembled. Ubiquitous expression of this gene is suggested by RT-PCR experiments, which have been carried out using RNA samples from several fetal tissues. Cosmid clones from within the critical region are currently being utilized for detection of submicroscopic deletions and cryptic translocations by fluorescent in situ hybridization (FISH).

Homologous Recombination (Joint)

U 008 EFFECTS OF LOSS-OF-FUNCTION MUTATIONS IN THE *PIM*-1 ONCOGENE *IN VITRO* AND *IN VIVO*, A. Berns, J. Domen, N. van der Lugt, M. van Lohuizen, H. te Riele, E. Robanus Maandag, C. Saris, P. Laird, A. Clark*, M. Hooper*, Division of Molecular Genetics of The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; *Department of Pathology, University Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, United Kingdom.

Transgenic mice overexpressing the *pim*-1 or *c-myc* proto-oncogene in their lymphoid compartment are predisposed to lymphomagenesis, although to a different extent. Infection of these mice with murine leukemia viruses results in the activation of other proto-oncogenes that collaborate with *pim*-1 or *c-myc* in lymphomagenesis. A number of additional genetic alterations contributing to tumor progression can be identified in this way. Recent findings with respect to some of these genes, such as *bmi*-1 and *pal*-1, will be discussed. In order to get insight into the normal function of these genes we have inactivated *pim*-1 and *bmi*-1 by homologous recombination in Embryonic stem cells. In doing so, we have analysed a number of factors that influence homologous recombination frequencies. We have studied in some detail mice lacking a functional *pim*-1 protein. They show no obvious phenotype. However, upon close examination of their hematopoietic compartment a number of effects were noted. Impaired growth factor responses were found both in mast cells and in lymphoid precursor cells. Implication for the function of *pim*-1 in signal transduction will be discussed.

U 009 TARGETED DISRUPTION OF THE MOUSE GLUCOCEREBROSIDASE GENE: DEVELOPMENT OF A MODEL OF GAUCHER DISEASE, Victor L.J. Tybulewicz[1], Michel L. Tremblay[2], Mary E. LaMarca[3], Barbara K. Stubblefield[3], Suzanne Winfield[3], Barbara Zablocka[3], Ellen Sidransky[3], Brian M. Martin[3], Sing-Ping Huang[2], Keith A. Mintze[1], Heiner Westphal[2], Richard C. Mulligan[1], and Edward I. Ginns[3], [1]Whitehead Inst for Biomed Res and Depart of Biology, MIT, Cambridge, MA 02142, and [2]Lab of Mam Genes and Dev, NICHD, Bethesda, MD 20892; and [3]Sec on Mol Neurogenetics, NSB, NIMH, Bethesda, MD 20892.

Gaucher disease, the most prevalent sphingolipidosis, results from the inherited deficiency of lysosomal glucocerebrosidase (GC). Despite its high frequency in humans, no animal model is available. A naturally occuring canine model of Gaucher disease was reported, but not propagated. Other attempts to create an animal model of Gaucher disease by inhibiting glucocerebrosidase in vivo have been inadequate. In order to produce mice having GC deficiency, we have disrupted the GC gene in mouse embryonic stem cells. The targeting plasmid for GC contained a neomycin gene inserted into exons 9 and 10 of GC, with an hsv-tk gene flanking the construct. This construct was introduced into ES cells by electroporation and the correct targeting event in doubly resistant clones was identified by Southern blot analysis. Four ES cell clones containing the targeted mutation in the GC gene in one allele were injected into blastocysts from C57BL/6 mice and transferred to FVB/N foster mice. One chimeric male transmitted the GC mutation to his progeny. A mouse strain carrying the mutation has been established. Mice homozygous for this "knockout" of the glucocerebrosidase gene have no detectable GC activity (<4% of control). These mice rapidly become cyanotic after birth, and have low weight, abnormal respiration, markedly decreased feeding and movement, and survive less than twenty-four hours. Histological analysis of affected mice shows massive infiltration of PAS positive lipid-laden cells in the liver, resembling Gaucher cells found in patients. Histological abnormalities, including PAS positive cells, are also present in the spleen, lung and bone. Although these mice have a rapidly progressive phenotype, they provide a model for the study of the pathogenesis of symptoms in patients with glucocerebrosidase deficiency. Glucocerebrosidase deficient mice with less severe phenotypes resembling the different types of Gaucher disease may be created either by introducing point mutations into the GC gene by homologous recombination, or by crossing transgenes carrying point mutations into the GC null mice described here. In this way we can obtain a model of Gaucher disease in which novel enzyme replacement, cellular transplantation and somatic gene transfer therapies can be evaluated.

Positional Cloning & Major Diseases-I (Joint)

U 010 MOLECULAR GENETICS OF CYSTIC FIBROSIS AND NEUROFIBROMATOSIS, Mitchell Drumm, David Gutmann, Theresa Strong, Douglas Marchuk, Lisa Smit, Paula Gregory, James Koh, Lone Andersen, Tom Sferra, Susan Wilson-Gunn, Dan Wilkinson, Anna Mitchell, David Lawson, and Francis Collins, University of Michigan, Ann Arbor, Michigan, 48109-0650

Positional cloning allows the identification of genes responsible for human disease without prior information on the normal function of the gene responsible for the disease. This strategy has recently yielded the genes for cystic fibrosis (CF) and neurofibromatosis (NF1). Research on these two diseases has now entered a different phase, which could be referred to as the "The Biology of Decoding". With the genes now in hand, investigations are now vigorously underway to deduce their normal function and to design better treatments for these two relatively common disorders.

Cystic fibrosis arises from mutations in the CFTR gene on chromosome 7; a consortium of laboratories working on this problem has identified more than 100 such mutations. Each of these mutations provides structure-function information about the CFTR protein. We are investigating mutant CFTR using a variety of expression systems, especially the Xenopus oocyte. Three days after RNA injection, wild type CFTR RNA leads to the production of a large chloride current which is cyclic AMP responsive. Surprisingly, the majority of other missense mutations (including ΔF508) have residual function in this assay, and can even be induced to generate near-wild type currents with high levels of phosphodiesterase inhibition. This result suggests a possible pharmacological approach to CF, which is being pursued by studying the same mutations in a variety of mammalian cells. In addition to these structure function analyses, a detailed investigation of the cis-acting sequences responsible for epithelial specificity of CFTR transcription is being pursued, and several potential regulatory sites have been identified by DNAse hypersensitivity, band shift analysis, footprinting, and expression analysis.

The full coding region of the NF1 gene has now been cloned and sequenced, and encodes a 2818 amino acid protein. In addition to the previously defined homology with GAP, other features of the gene are being actively studied. We have raised antisera against several domains of NF1, and using immunofluorescence demonstrated that the NF1 protein co-localizes with microtubules. This unexpected observation suggests that signal transduction mediated by NF1 may involve the cytoskeleton. A variety of mutations have also been identified in the NF1 gene which are associated with disease, and a vigorous search is being undertaken for evidence for mutation in this gene in tumors from patients who do not have NF1, though the large size of the gene makes such analysis fairly difficult.

U 011 MICRODELETIONS OF 22q11.2: A GENETIC ETIOLOGY FOR DiGEORGE SYNDROME, VELO-CARDIO-FACIAL (VCF) SYNDROME AND CHARGE ASSOCIATION. Beverly S. Emanuel[1], Deborah A. Driscoll[1], David Ledbetter[2], Fangrong Zhang[2] and Marcia L. Budarf[1], [1]The Children's Hospital of Philadelphia. Philadelphia, PA 19104 and [2]Baylor College of Medicine, Houston, TX.

DiGeorge Syndrome (DGS) is a developmental defect of the 3rd and 4th pharyngeal pouches. The disorder is characterized by aplasia or hypoplasia of the thymus and parathyroid glands and conotruncal cardiac malformations. Cytogenetic data suggests that the DGS critical region (DGCR) lies within 22q11.2. We have used dosage studies of probands and RFLP analysis of proband and parents to begin to define the DGS critical region in 14 patients. DNA Deletions have been detected in 5 DGS patients with visible interstitial deletions, del(22)(q11.21q11.23). Probands for whom cytogenetic studies were not unequivocally diagnostic of a deletion also have DNA deletions. In addition, microdeletions of 22q11 have been detected in 6 of 6 cytogenetically normal DGS probands. The minimal region of overlap for these fourteen patients includes loci D22S75 (N25), D22S66 (pH160b) and R32. Together these probes recognize a minimal region of 500 kb. The region is flanked proximally by locus D22S36(pH11) and distally, by BCRL2. By RFLP analysis in 5 families, 4 of 5 probands failed to inherit a maternal allele; the 5th proband failed to inherit a paternal allele. Based on these data and our observation of maternally and paternally inherited translocation-derived DGS probands, there does not appear to be a consistent parent of origin for DGS associated deletions of chromosome 22. Five additional DGS patients have been studied using at least one of the three commonly-deleted probes and they all have demonstrated 22q11.2 DNA deletions. Thus, 19/19 DGS patients show deletions of 22q11.2. YACs for probe N25 have been isolated and have been successfully utilized for detection of deletions by fluorescence *in situ* hybridization (FISH) techniques. Several other disorders, Velo-cardio-facial syndrome (VCF or Shprintzen) and the CHARGE association, are syndromes in which the the abnormalities which constitute the DiGeorge anomalad also play a significant role. We have detected N25 deletions in 5 patients with VCF syndrome; 2 by dosage analysis and 3 by FISH. Similarly, N25 deletions have been detected in 2 patients with the CHARGE association by dosage analysis. These data suggest a genetic etiology, deletion of 22q11.2, for all three disorders and support the combined use of cytogenetic and molecular analysis for the diagnosis of DGS, VCF and CHARGE association Progress using the YACs for analysis of the DGCR will be discussed.

U 012 POSITIONAL CLONING APPROACH TO POLYCYSTIC KIDNEY DISEASE, Stephen T. Reeders[1,2,3], Gregory G. Germino[1], Stefan Somlo[1], Debra Weinstat-Saslow[2], Marita Pohlschmidt4, and Anna-Maria Frischauf[4].[1]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510; [2]Department of Genetics, Yale University School of Medicine, New Haven; [3]Howard Hughes Medical Institute, Yale, New Haven; [4]Imperial Cancer Research Fund Labs, London.

The most common form of autosomal dominant polycystic kidney disease (ADPKD) has been mapped to chromosome 16p13.3. The 500kb region in which the disease gene, PKD1, lies has been defined by flanking genetic markers. An overlapping set of ~40 genomic cosmid clones spanning the entire region has been isolated. Restriction mapping suggested that the region was packed with CpG islands. Genes corresponding to almost all of these islands have been identified by cDNA cloning.

No major rearrangements have been identified in the genes in the PKD1 region. Therefore a sequence-based approach is required to screen the ~20 genes from the region. The alternative strategies for mutational screening and the prioritization of PKD1 candidates based on likely function will be discussed.

Positional Cloning & Major Diseases-II (Joint)

U 013 CYSTIC FIBROSIS: PROBING THE BASIC DEFECT, Lap-Chee Tsui[1,2], Danuta Markiewicz[1], Julian Zielenski[1], Ting-Chung Suen[1], Richard Rozmahel[1,2], Mark Dobbs[1], John Teem[1], Joseph Chien[1], Martha Glaves[1], Venus Lai[1], Mary Corey[3], Sasha Dho[4], Kevin Foskett[4], Peter Durie[3,5,6], and Johanna Rommens[1], Department of [1]Genetics and [3]Pediatrics, Divisions of [4]Cell Biology and [5]Gastroentorology, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, and Departments of [2]Medical Genetics and [6]Pediatrics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Cystic fibrosis (CF) is a common autosomal recessive disorder in the Caucasian population. The mutated gene causing this disease was identified on the basis of its chromosomal localization by linkage analysis. As a basic defect in CF involved the regulation of a cAMP-inducible chloride permeability in the apical membrane of secretory epithelial cells, the encoded polypeptide was named *Cystic Fibrosis Transmembrane Conductance Regulator* (CFTR).

Expression of CFTR appears to be specific to the tissues that are affected in CF patients, e.g. lung, pancreas, intestine, liver, sweat gland and nasal epithelia. The primary sequence shows that this1480-amino-acid CFTR polypeptide contains 2 almost identical halves, each containing a membrane-spanning region followed by an ATP-binding domain. In order to delineate the basic defect in CF, four different lines of experiments are being pursued in our laboratory:

1. Identification of mutations in the CFTR gene. The major mutation causing CF is a 3-bp deletion (named ΔF508) removing a phenylalanine residue from the first ATP-binding domain of CFTR; it accounts for approximately 70% of the mutant chromosomes. To facilitate identification of the remaining mutations and to coordinate screening of population frequencies for each mutation, a worldwide consortium has been formed. Over 150 different mutations have been reported; most of them are rare and their frequencies vary among different ethnic groups. The data also support the predicted roles for the various domains of CFTR and provide a molecular basis for future structural analysis of the protein.
2. Correlation of genotype and phenotype. The concordance of pancreatic function among patients within the same family suggests that this clinical phenotype is predisposed by mutation in the CFTR gene. Two groups of mutant alleles are evident after examining over 500 CF patients in our Hospital. Patients carrying one or two *mild* mutant alleles are invariably pancreatic sufficient (PS) whereas patients with two *severe* mutations (such as ΔF508) are almost certainly pancreatic insufficient (PI). The *severe* alleles include nonsense, frame-shift, splicing mutations and missense mutations, some of which affecting the highly *conserved residues within the ATP-binding domain. Besides the fact that all* seven *mild* alleles identified are amino acid substitutions, the majority of them are located in the predicted transmembrane regions of CFTR. Possible correlation between genotype and other clinical symptoms are being further investigated.
3. Direct assay of CFTR function. Full-length CFTR cDNA clones have been generated and introduced into various cell types for functional analysis. Although the overall structure of CFTR resembles some of the prokaryotic and eukaryotic *transport proteins, the results of numerous DNA transfection studies strongly* suggest that CFTR itself functions as a small conductance chloride channel that is inducible by intracellular cAMP.
4. Model systems. In order to gain further insight into the pathophysiology of CFTR mutations *in vivo*, we have initiated attempts to create a mouse model for CF by trying to disrupt the mouse Cftr gene via homologous recombination in embryonic stem cells. Minigene constructs are also being generated to recreate the human mutations. *In addition, we have inserted a segment of the first ATP-*binding domain of the human CFTR into the corresponding region of the yeast STE6 protein but retained its transport activity for the *a*-mating factor. This activity can be destroyed by the introduction of the ΔF508 mutation into the CFTR segment and subsequently restored by second site mutations.

U 014 EXPRESSION OF NATURAL AND RECOMBINANT HUMAN DYSTROPHIN GENES. Ronald G. Worton, Henry J. Klamut, Lucine O. Bosnoyan, Christine Tennyson, Xiuyuan Hu and Peter N. Ray, Genetics Department and Research Institute, Hospital for Sick Children, and Department of Molecular and Medical Genetics, University of Toronto, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8.

Dystrophin is a high molecular weight cytoskeletal protein localized at the inner surface of the sarcolemmal membrane of skeletal muscle. Its function is not well understood, but its deficiency is responsible for the progressive degeneration of muscle in boys with Duchenne muscular dystrophy (DMD). Two outstanding problems in DMD research are to understand the function of dystrophin and to devise effective therapy for the disease. Genetic manipulation of the dystrophin molecule is one approach to the former and dystrophin replacement is a logical approach to therapy. Both types of study may benefit from the generation of recombinant dystrophin minigenes that can be genetically altered and expressed in cultured myogenic cells and in the skeletal muscle of affected boys. One potentially important aspect of such studies is the regulation of the dystrophin gene in the myogenic environment. To better understand dystrophin gene regulation we had previously isolated, *sequenced and characterized the muscle-specific promoter of the dystrophin* gene, defining a minimal promoter of 150 bp of upstream sequence. Within this we identified regions of homology to previously defined muscle-specific regulatory elements such as a CarG box and MEF-1 (MyoD) binding sites, and demonstrated tissue-specific and differentiation-specific expression. More recently studies with reporter gene constructs have revealed within the minimal promoter both positive and negative regulatory regions, and in intron 1 of the gene a muscle-specific enhancer. Other factors determining dystrophin level in muscle include efficiency of message elongation and stability of the message. The extreme size of the dystrophin gene (2300 kb) points to potential problems in completing transcripts and suggests a time to complete a transcript in excess of 20 hours. To investigate these factors, primer pairs that detect mature message at both the 5' and 3' end of the gene have been used to study message accumulation in human fetal muscle cells following fusion into multi-nucleated myotubes. The rate of accumulation of 3' sequences is considerably less than that for 5' sequences, suggesting premature termination or pausing during message elongation. Since, sequences in the 5' and 3' untranslated region (UTR) of several genes have been shown to affect message stability and translational efficiency. Sequences from the highly conserved 3' UTR of the dystrophin gene are being tested for their effect on expression of a growth hormone *reporter gene. Finally*, as a prelude to dystrophin functional studies, and to potential gene therapy, a functional dystrophin minigene has been constructed and expressed in myogenic cell culture. The gene is reduced in size by a 5.3 kb in-frame deletion that removes much of the central spectrin-like repeat domain from the protein, but dystrophin localizes properly in transfected myotubes. Additional constructs are being tested for membrane localization and for expression in transgenic *mdx* mice.

Gene Transfer/ Gene Therapy-I (Joint)

U 015 APPLICATIONS OF MURINE GENE TARGETING TO HUMAN GENETICS, Arthur L. Beaudet, Raymond W. Wilson, James E. Sligh, Christie M. Ballantyne, Wanda K. Lemna, Gerald Patejunas, E. Paul Hasty, Allan Bradley and William E. O'Brien, Howard Hughes Medical Institute and Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

We envision at least three applications of murine gene targeting for analysis of human genetic disease. First, mouse models of known human disorders can be generated with emphasis on studies of pathophysiology and therapy, particularly if the mouse phenotype is very similar to the human phenotype. If the mouse and human phenotypes are distinctly different, attempts to understand the basis of this difference still may shed light on pathophysiology and suggest therapeutic strategies. Second, mutations can be generated in genes where no mutants are yet identified in mammals. Analysis of the murine phenotype may allow for recognition of the molecular defect in homologous human disorders. Third, animals with mild phenotypes in heterozygotes or homozygotes can be used to analyze the role of candidate genes in polygenic disease processes. Following these rationales, gene targeting has been attempted in our laboratory for argininosuccinate synthetase (AS), cystic fibrosis (CF), CD18, and ICAM-1. Gene targeted clones were identified and chimeric mutant animals obtained for AS, CD18, and ICAM-1. Germline transmission of a hypomorphic allele was achieved for CD18, and homozygotes are fertile and demonstrate a mild phenotype. Homologous recombination was many orders of magnitude less frequent for the CF locus. Mutations in AS, CD18, and CF should provide mouse models for known human genetic disorders. Mutations in CD18 and ICAM-1 will be examined for polygenic effects on autoimmune processes, atherosclerosis, transplantation rejection, and other inflammatory processes.

U 016 Hepatic Gene Therapy in Animal Models. Savio L.C. Woo, Howard Hughes Medical Institute, Departments of Cell Biology and Molecular Genetics, Baylor College of Medicine, Houston, TX.

The liver is the major organ for metabolism and there are dozens of known metabolic disorders secondary to a variety of hepatic deficiencies in man. The development of technologies to deliver functional genes into hepatocytes in vivo would permit gene therapy for these disorders in the future. In our laboratory, two deficient animal models are being tested for the efficacy of gene therapy approaches for their phenotypic correction. Phenylketonuria (PKU) is an inborn error in amino acid metabolism that causes severe and permanent mental retardation in affected children. The genetic disorder is secondary to a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) which converts phenylalanine to tyrosine and constitutes the major metabolic pathway for the essential amino acid. We have previously reported the cloning of the human PAH cDNA and the use of it to express the human enzyme in heterologens mammalian cells by retroviral mediated gene transfer. More recently, we have also demonstrated that heterologous hepatocytes injected directly into the portal vein or the spleen of cogenic mice migrated to the liver, survived and continued to function as hepatocytes for the life of the recipients. Independently, a PAH-deficient mouse model has been created by the Laboratory of William Dove at the University of Wisconsin. Thus, we have initiated collaborative studies to attempt the correction of the deficient phenotype in the mutant mouse model. Virally transduced hepatocytes expressed high levels of human PAH, and these cells will be transplanted into deficient animals in order to determine if there will be long-term reconstitution of the enzyme activity in the liver. The second animal model is the Factor IX-deficient dog in the laboratory of Kenneth Brinkhous at the University of North Carolina in Chapel Hill. This is a severe bleeding animal model and the construction of recombinant retroviruses expressing human and canine Factor IX in fibroblasts and primary hepatocytes has previously been reported. We have recently established methods for the isolation of $3x10^9$ hepatocytes from a single liver lobe obtained from 5 kg dogs by partial hepatectomy, as well as autologous transplantation of these cells back into the same animal via injection into the portal vasculature. After retroviral transduction and transplantation, the cells survived for a minimum of 4 months in vivo. If successful, these technologies can be directly applied to the treatment of a variety of metabolic disorders in man in the future.

Gene Transfer/ Gene Therapy-II (Joint)

U 017 HIGH EFFICIENCY GENE TRANSFER INTO HEMATOPOIETIC PRECURSORS, C. Thomas Caskey, Kohnoske Mitani and Annemarie Moseley, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

Adenosine deaminase (ADA) deficiency, a recessively inherited disease, accounts for 15% of severe combined immunodeficiency cases. Therapies include HLA-matched bone marrow transplantation, replacement with PEG-ADA and retroviral transduction of peripheral lymphocytes with the human ADA gene. While often associated with morbidity, transplantation with HLA-matched offers a potential cure, making ADA an ideal candidate for bone marrow gene therapy. Previously, we have demonstrated transduction of the hADA gene into bone marrow from normal donors and ADA deficient patients by means of co-cultivation with the virus-producing cell line, AM12st14, in the presence of rhIL3 and rhIL6. Infection efficiency, as determined by PCR detection of provirus integration into myeloid colonies grown in methylcellulose culture (CFU-GM), is 34% after 9 weeks in long-term culture. Since committed clonogenic precursors are not maintained in culture longer than 4-5 weeks, the data demonstrated that successful transduction of hematopoietic precursors was possible. Expression of the hADA gene in the CFU-GM was determined by RT-PCR and 50% of the CFU-GM which had integrated the provirus expressed the transduced gene. Microradioassay for adenosine deaminase activity correlated with mRNA expression. Subsequently we have focused on infection with supernatant from the AM12st14 cell line. We have achieved supernatant infection of normal bone marrow cells which were separated on Percoll gradient and were then maintained in culture for 2-3 weeks prior to infection. Supernatant was added every 12 hours in the presence of rhIL3 and rhIL6 for 72 hours. Proviral integration was present in 70% of the colonies at 7 weeks post-infection (*i.e.*, 10 weeks in LTC) and significant levels of transcript were seen. The presence of the stromal layer was essential for supernatant infection. We have expanded these studies to include the infection of a population of bone marrow cells enriched for more primitive hematopoietic precursors. Autologous stromal layers are initially developed from the low density Percoll gradient fraction. The remaining cells are cryopreserved until the stromal layers are nearly confluent, at which time the cells undergo positive selection for CD34+ cells using anti-CD34 coated flasks (Applied Immune Sciences). Infection efficiency is nearly equivalent to that seen with co-cultivation. The ability to infect the primitive hematopoietic progenitor containing population with retroviral supernatant greatly facilitates the clinical introduction of bone marrow gene therapy.

U 018 GRAFTING GENETICALLY MODIFIED CELLS TO THE BRAIN, Fred H. Gage, Department of Neurosciences, University of California, San Diego.

To assess the feasibility of using primary skin fibroblasts as a donor population for genetic modification and subsequent intracerebral grafting, we examined the structural and neurochemical characteristics of grafts of isogeneic primary fibroblasts over a period of six months. Following the implantation in adult rats from the same inbred strain, isologous grafts are stained immunohistochemically for fibronectin immunostaining which persists for at least six months. Immunostaining for laminin is intense within the grafts from one to eight weeks, but decreases by six months. Astrocytes respond dramatically to the implantation of primary fibroblasts although the intensity of immunostaining for glial fibrillary acidic protein diminishes between eight weeks and six months. The astrocytic border between the grafts and striatal neuropil remains intensely immunostained. Capillaries within the grafts stain immunohistochemically for glucose transporter as early as three weeks after implantation. At the ultrastructural level, grafts possess numerous fibroblasts and have an extracellular matrix filled with collagen. Reactive astrocytic processes filled with intermediate filaments are found throughout the grafts. Hypertrophied astrocytes and their processes also appear to form a continuous border between the grafts and the striatal neuropil. Grafts of primary fibroblasts also possess an extensive vasculature that is composed of capillaries with nonfenestrated endothelial cells; the occurrence of reactive astrocytic processes closely associated with or enveloping capillaries is variable. These results provide direct morphological and neurochemical evidence for the long-term survival of isologous fibroblasts after intracerebral implantation. We have transduced primary isologous fibroblasts with several different transgenes to assess the effectiveness of these genetically modified cells to delivery new genetic material to the brain, including Nerve Growth Factor (NGF), Tyrosine Hydroxylase, GABA Decarboxylase, and Choline Acetyltransferase. We will report on intracerebral grafts of primary skin fibroblasts genetically engineered to expressing several of these transgenes, including NGF which had been embedded within a collagen matrix prior to grafting. This later experiment was designed to assess the regenerative capacity of cholinergic neurons of the adult rat medial septum. The results reveal the following: first, NGF-producing grafts sustain a significant number of NGF receptor-immunoreactive septal neurons following axotomy. Second, NGF promotes the regeneration of septal axons, such that NGF-producing grafts possess large numbers of unmyelinated axons which use many a variety of substrates for growth. Grafts of control fibroblasts possess the same cellular and matrix substrates but contain only a very small population of axons. Advantages and disadvantages of these grafted genetically modified primary fibroblasts will also be discussed.

U 019 GENE THERAPY OF FAMILIAL HYPERCHOLESTEROLEMIA, Mariann Grossman and James M. Wilson, Departments of Internal Medicine and Biological Chemistry, and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan.

Familial Hypercholesterolemia (FH) is an autosomal dominant disorder in humans caused by a defect in LDL receptor expression. Homozygous patients have severe hypercholesterolemia and premature coronary heart disease. We are developing two approaches for treating FH patients based on liver directed transfer of the LDL receptor gene. An animal model for FH called the WHHL rabbit has been used to demonstrate the feasibility and efficacy of our approaches. One strategy, involves the transplantation of autologous hepatocytes genetically corrected ex vivo with recombinant retroviruses. The feasibility and long-term efficacy of this approach has been demonstrated in the WHHL rabbit. Issues of safety have been addressed in experiments performed with dogs and baboons. Human trials should begin in 1992.

Another approach to gene therapy of FH is to construct a gene transfer substrate capable of targeting to hepatocytes when infused into the peripheral circulation. This substrate is formed by complexing a ligand to a hepatocyte specific receptor with a transfection-based, LDL receptor-expressing vector. When infused into the peripheral circulation of WHHL rabbits, the DNA/protein complex is targeted to hepatocytes and transiently expressed leading to a temporary improvement of hypercholesterolemia.

Genomic Imprinting

U 020 GENETIC STUDIES UPON CHROMOSOME IMPRINTING IN MICE, Bruce M. Cattanach, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, UK.

Pronuclear transplantation experiments with mice have suggested that both a male and female genome may be necessary for normal embryonic development. Genetic studies using a variety of Robertsonian and reciprocal translocations have however shown that it is not the whole genome that is involved. Rather it is only genes in 9 regions of 5 different chromosomes for which parental origin is important. Parental imbalance for each of the regions, as in maternal and paternal disomies, results in anomalies of development. With imbalance for some regions early embryonic death ensues, with others, deaths occur pre-natally, and with yet others, anomalies of post-natal development are found. The concept to explain these effects is that certain genes are imprinted or marked in the parental germ lines with the consequence that maternal and paternal copies are differentially expressed in the embryo. Maternal and paternal disomies therefore have an excess or deficiency of gene products, and through the identification of three imprinting genes direct evidence of this has been obtained. It is not yet clear whether only one or a series of genes are responsible for the imprinting anomalies associated with each chromosome region. Current evidence suggests that only few genes are involved and that these may be growth factors or receptors, or otherwise have important functions in embryonic development. The mechanisms responsible for the differential gene expression have not yet been elucidated.

U 021 ADULT PHENOTYPE IN THE MOUSE CAN DEPEND ON EPIGENETIC EVENTS IN THE EARLY EMBRYO, Wolf Reik, Sarah K. Howlett, Sheila C. Barton, M. Azim Surani, Lucy Bowden, Irmgard Gurtmann[1] and Joachim Klose[1], Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge and [1]Department of Human Genetics, University of Berlin

A number of observations in the mouse suggest that there are nucleocytoplasmic interactions in the early embryo that can affect epigenetic programming of later gene expression. It is possible that some influences of parental inheritance on offspring phenotype are caused by this type of mechanism. We have begun to examine the effect of parental inheritance of alleles on offspring phenotype by analysing 2-dimensional protein patterns in adult livers of reciprocal F1 hybrids between C57BL/6 and DBA/2. To assess the effect of correct nucleocytoplasmic interaction on adult phenotype, experimental zygotes were made that have a B6 type cytoplasm, DBA type maternal chromosomes and B6 type paternal chromosomes. Hence, the usual genotypic harmony between cytoplasm and maternal genome has been disrupted in these nucleocytoplasmic hybrids. Proteins were extracted from adult liver of these animals and their 2D electrophoresis patterns were compared to those of the parental strains and the natural F1 hybrids. A prominent group of liver proteins was found whose expression was virtually undetectable in the nucleocytoplasmic hybrids. A partial amino acid sequence was obtained of two members of this group. The amino acid sequence identified these polypeptides as belonging to the Major Urinary Protein (MUP) family. Some liver specific MUP genes were found to be highly methylated in the nucleocytoplasmic hybrids as compared to normal controls. Transcription of the methylated MUP genes was substantially reduced, suggesting that most of the MUP repression in BDB hybrids is at the transcriptional level. Our results suggest that there may be specific nucleocytoplasmic interactions in the early embryo that programme the genome for future function. Hence, aspects of adult phenotype can depend upon epigenetic events in the early embryo.

U 022 GENOMIC IMPRINTING AND MAMMALIAN DEVELOPMENT, Davor Solter[1], Keith Latham[2], and Julie A. DeLoia[2], [1]Max-Planck Institute of Immunobiology, 7800 Freiburg, Germany, [2]The Wistar Institute, Philadelphia, PA 19104.

Genomic imprinting in mammals results in functional differences in the oocyte and spermatocyte genomes. Such functional differences affect various aspects of development and gene expression. Analysis of the developmental capacities of androgenetic and gynogenetic mouse embryos (bearing two paternal or two maternal pronuclei, respectively) revealed a defect in blastocyst formation of androgenetic, but not gynogenetic, embryos that was a function of the maternal genotype. Androgenetic embryos constructed using fertilized eggs from C57BL/6 or (B6D2)F_1 mice developed to the blastocyst stage at frequencies similar to those previously reported, whereas androgenetic embryos constructed with fertilized eggs from DBA/2 mice developed poorly, the majority failing to progress beyond the 16-cell stage and unable to form a blastocoel-like cavity, regardless of whether the male pronuclei were of C57BL6 or DBA/2 origin. Several specific alterations in the protein synthesis pattern of DBA/2 androgenomes were observed that reflect a defect in the regulatory mechanisms that normally modulate the synthesis of these proteins between the 8-cell and blastocyst stages. These results are consistent with a model in which cytoplasmic factors present in the egg direct a strain-dependent modification of paternal genome function in response to epigenetic modifications (genomic imprinting) established during gametogenesis and indicate that preimplantation development can be affected by these modifications at both the morphological and biochemical levels. We also describe a transgenic mouse line, Adp, in which the transgene has integrated into a functionally imprinted region of the genome. Transmission of the transgene through the paternal lineage is associated with paw and skull deformities in the offspring, whereas maternal transmission does not interfere with normal development. The mutation caused by the transgene insertion shows variable expressivity and incomplete penetrance, and a modified dominant pattern of inheritance. Cosmid clones flanking the transgene insertion site were used for chromosomal localization by RFLP analysis in BXD recombinant inbred strains. This analysis mapped the transgene to proxial mouse chromosome 7, in a region where maternal duplication and paternal deficiency are lethal. The results of this analysis are consistent with the transgene imprinting pattern. This region is homologous with HSA 19q, which includes the locus for DM, a human disorder affected by imprinting. Cosmids spanning the integration site have been isolated and screened for regions which have been conserved between species and for transcriptionally active DNA segments. Study of the Adp lineage should provide insight into the molecular mechanisms involved in the imprinting process.

Eye Disorders

U 023 GENETIC HETEROGENEITY IN AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA, Shomi S Bhattacharya[1], Rumaisa Bashir[1], Jeffrey Keen[1], Marcelle Jay[2], Alan Bird[2], and Chris Inglehearn[1], [1]Department of Human Genetics, University of Newcastle upon Tyne, UK. [2]Department of Clinical Ophthalmology, Moorfields Eye Hospital, London, UK.

Retinitis Pigmentosa is clinically a heterogeneous group of inherited retinal degenerations following X-linked, autosomal dominant and autosomal recessive patterns of inheritance. The incidence of all types of RP is 1/3000. The disease primarily affects the rod photoreceptor cells of the retina leading to night blindness, pigmentary patches on the retina, and ultimately to total blindness. Clinically autosomal dominant Retinitis Pigmentosa (adRP) has been divided into two types: D-type/type I with early onset and R-type/type II with variable age of onset. In D-type the photoreceptor function loss shows a diffuse pattern whereas in R-type it is regional. Molecular genetic linkage studies have led to the identification of 3 loci responsible for adRP. The gene encoding rhodopsin (RHO), the rod photopigment, which maps to chromosome 3q, has now been shown to be mutated in approximately 30% of adRP patients (Sung et al 1991[1], Dryja et al 1991[2], Keen et al 1991[3]). We found 12 British adRP families with RHO mutations, out of a completed screen of 39 families. This screen revealed correlation between phenotype and genotype, since all RHO-RP families found were in the D-type or sectorial RP categories, while two large families excluded from the region by linkage analysis are R-type in phenotype. More recently two new adRP loci have been reported. Blanton et al (1991)[4] obtained a two point lodscore of 10.2 at $\hat{\theta} = 0.07$ with chromosome 8p marker PLAT in a large Louisiana adRP pedigree. Also two groups have reported mutations in the human Rds gene (known to be the site of the 'Retinal Degeneration Slow' mutation in mouse) in adRP patients (Farrar et al 1991[5], Kajiwara et al 1991[6]). However linkage data in our two R-type non-rhodopsin families exclude both these loci, demonstrating that adRP can result from mutations at at least four loci.

1) Sung C-H, Davenport CM, Hennessey JC, Maumenee IH,Jacobson SG, Heckenlively JR, Nowakowski R, Fishman G, Gouras P and Nathans J. (1991) Rhodopsin mutations in autosomal dominant Retinitis Pigmentosa. Proc Natl Acad Sci USA 88: 6481-85. 2) Dryja TP, Hahn LB, Cowley GS, McGee TL and Berson EL. (1991) Mutation sprectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. Proc Natl Acad Sci USA 88: 9370-74. 3) Keen TJ, Inglehearn CF, Lester DH, Bashir R, Jay M, Bird AC, Jay B and Bhattacharya SS. (1991) Autosomal dominant retinitis pigmentosa: four new mutations in rhodopsin, one of them in the retinal attachment site. Genomics 11: 199-205. 4) Blanton SH, Heckenlively JR, Cottingham AW, Friedman J, Sadler LA, Wagner M, Freidman L and Daiger SP. (1991) Linkage mapping of autosomal dominant retinitis pigmentosa (RP1) to the pericentric region of human chromosome 8. Genomics 11: In press. 5) Farrah GJ, Kenna P, Jordan S, Singh RK, Humphries MM, Sharp EM and Humphries P. (1991) A three base pair deletion in the peripherin - rds gene in one form of retinitis pigmentosa. Nature. In Press. 6) Kajiwara K, Hahn LB, Mukai S, Travis GH, Berson EL and Dryja TP. (1991) Mutations in the human retinal degeneration slow gene (rds) in autosomal dominant retinitis pigmentosa. Nature. In Press.

U 024 MOLECULAR CLONING OF THE cDNA FOR A NOVEL PHOTORECEPTOR-SPECIFIC MEMBRANE PROTEIN (ROM-1) IDENTIFIES A DISK RIM PROTEIN FAMILY IMPLICATED IN HUMAN DEGENERATIVE RETINOPATHIES. Roderick R. McInnes, Roger A. Bascom, Robert S. Molday and V. I. Kalnins; Dept. of Genetics, Research Institute, Hospital for Sick Children and Depts. of Molecular and Medical Genetics and Anatomy, University of Toronto, 555 University Ave. Toronto, Ontario Canada M5G 1X8, and Dept. of Biochemistry, Univ. of British Columbia, Vancouver, B.C. V6T 2B5.

The opsin-containing disks of the outer segments of vertebrate photoreceptors are continually renewed by disk morphogenesis and shedding (at a rate of ~10% per day), but these processes are largely uncharacterized at the molecular level. In addition, the outer segments have been shown to be the primary site of pathology in several mammalian hereditary degenerative retinopathies, such as autosomal dominant retinitis pigmentosa (ADRP) due to mutations in the rhodopsin gene. We hypothesized that evolutionarily conserved genes that are expressed abundantly and specifically in the retina are likely to be important to its structure and function, and to constitute candidate genes for retina-specific diseases. To identify such genes, we used differential hybridization to select conserved, abundant, and retina-specific cDNA clones.

One cDNA identified by this strategy encodes a 37 kDa integral membrane protein which we have localized to the outer segment of the rod photoreceptors. This protein, designated rom-1, is 35% identical to peripherin, the protein encoded by the human retinal degeneration slow (*RDS*) gene. The overall similarity of structure (size, sequence and hydrophobicity) of rom-1 and peripherin suggests that they define a new photoreceptor-specific protein family. In addition to being structurally related, the rom-1 and peripherin proteins are biologically similar. Both are located at the rod disk rims, where they may serve to link the disks to the plasma membrane. In addition, the two proteins have similar membrane topology, and exist as dimers *in vivo*. Although they -do not form heterodimers with each other, they may be non-covalently associated *in vivo*, since rom-1 completely and specifically co-purifies with immunoabsorbed peripherin on an affinity column.

The many similarities between rom-1 and peripherin, together with the phenotype of the degenerative retinopathy in the *rds* mouse and the recent identification of mutations in the human *RDS* gene in patients with ADRP (T. Dryja; P. Humphries, 8th Intl. Congress of Human Genetics), suggest that both proteins play important roles in the morphogenesis of the rod outer segments, and that *ROM1* is also a strong candidate gene for human retinopathies. Notably, all three substitutions described by Dryja in the *RDS* gene in ADRP patients affect residues located in domains perfectly conserved between rom-1 and peripherin. To facilitate the search for disease association, the *ROM1* gene has been mapped in man (chr 11q13) and mouse (chr 19). The 1 kb coding region of the human *ROM1* gene is interrupted by only two introns (385 and 116 bp). The small size of the gene has facilitated its analysis by SSGE and direct sequencing in patients with ADRP who do not have mutations in rhodopsin. To date, in 122 unrelated patient alleles partially screened by SSGE, one common polymorphism (in intron 1) and three amino acid substitutions have been identified. The relationship between the substitutions and the ADRP in the affected individuals is being examined by segregation analysis and biochemical characterization.

U 025 A CANDIDATE GENE FOR THE OCULOCEREBRORENAL (LOWE'S) SYNDROME, Olivier Attree[1], Ichiro Okabe[1], Isabelle Olivos[1], L. Charles Bailey[1], David L. Nelson[2], Richard A Lewis[2], Roderick McInnes[3], Robert Nussbaum[1], [1]Howard Hughes Medical Institute and Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, [2]Baylor College of Medicine, Houston TX, [3]Hospital for Sick Children, Toronto, Ontario, Canada.

The Oculocerebrorenal Syndrome OCRL (Lowe Syndrome) is an X-linked disorder characterized by congenital cataracts, neonatal hypotonia, severe mental retardation, and a proximal renal tubular defect (aminoaciduria, phosphaturia, proximal renal tubular acidosis). The basic biochemical defect responsible for this disorder is unknown. The OCRL locus was mapped to the Xq25-q26 region by linkage analysis [1] to RFLP markers DXS42, DXS100, HPRT, DXS177, DXS10 and DXS86 and more accurately by the identification of two female patients with severe OCRL, one with an X;3 translocation [2] and the other with an X;20 translocation [3], both involving a region of the X chromosome at the boundary between Xq25 and Xq26. As part of large scale project to map yeast artificial chromosome (YAC) clones containing X chromosome inserts, two YAC clones (RS88 and RS41) were mapped to the region of the OCRL locus and were found to span the breakpoint in the female patient with an X;3 translocation [4]. These two YACs were used to screen directly cDNA libraries of bovine lens and human kidney [5]. Two distinct classes of cDNA were identified: the first set of cDNAs, obtained from lens, kidney, brain, and fibroblast, detects a ~4.5 kb transcript which is not disrupted by the X;autosome translocations in females with OCRL, is normal in fibroblast RNA from 12 OCRL patients, and has regions of strong homology to the S. cerevisiae SNF2 gene. The other cDNAs detect a ~5.5 kb transcript expressed in kidney, brain, and fibroblast that is disrupted by the X;autosome translocations in females with OCRL and does detect abnormal or absent transcripts in six of 12 male patients with OCRL and no detectable abnormality on Southern blot. This cDNA contains an open reading frame of 852 amino acids that bears no homology to sequences in GenBank and has no distinctive motifs to provide a clue as to its function. The identity and function of the encoded protein is currently under investigation in normals and OCRL patients as a reasonable candidate gene for OCRL.

1. Reilly DS, Lewis RL, Nussbaum RL. Genom 8:62-70, 1990.
2. Hodgson SV, Heckmatt JZ, Hughes E, et al. Amer J Med Genet 23:837-847, 1986.
3. Mueller OT, Hartsfield MK, Gallardo LA, et al. Am J Hum Genet 49:804-810, 1991.
4. Nelson, D.L, Ballabio, A., Victoria, M.F. et al. Proc Natl Acad Sci USA 88 (1991) 6157-6161.
5. Okabe I, Attree O, Bailey LC, et al. J Inherited Metabolic Disease, in press.

U 026 POSITIONAL CLONING OF GENES FOR X-LINKED BLINDNESS, DEAFNESS AND MENTAL RETARDATION. Irene Bach, Wolfgang Berger[1], Han Brunner[1], José van den Hurk[1], Alfons Meindl[2], Thomas Meitinger[2], Marcus Pembrey[3], Christophe Philippe[4], Dorien van de Pol[1], Frans Cremers[1], and H. Hilger Ropers, [1]Department of Human Genetics, University of Nijmegen, [2]Department of Pediatrics, University of Munich, [3]Institute of Child Health, University of London, [4]Laboratory of Genetics, CRTS, Nancy

Choroideremia, mixed deafness with or without stapes fixation and mental retardation are consistent clinical features of male-viable large deletions involving the Xq21 band. Characterization of several large and small Xq21 deletions has enabled us to precisely define submicroscopic intervals carrying the genes for these disorders and to clone a candidate gene for choroideremia (CHM). Mutation screening in unrelated patients has revealed numerous different stop codon or frame shift mutations thereby providing unambiguous evidence for the causal role of this gene in choroideremia. Sequence homology with GDI, a gene involved in the regulation of the GDP exchange of the small G protein smg p25A, suggests that the choroideremia gene product interacts in a similar way with G proteins of the retina or choroid. CHML, a recently detected homologue of CHM which maps on chromosome 1q in the same region as the Usher 2 gene, is likely to have a similar function.

In order to narrow down the intervals on proximal Xq that carry the genes for deafness (DFN) and mental retardation (MR), we have performed a systematic search for microdeletions in patients with non-syndromic, non-fra(X) X-linked MR and in patients with X-linked DFN. In two DFN patients, microdeletions were detected with probe pHU16 (DXS26) which are being characterized by long range physical mapping and YAC analysis. Moreover, probe screening, radiation-reduced hybrids and Alu-PCR have enabled us to map the breakpoint on the proximal Xq in a mentally retarded girl with a de novo X-autosome translocation. Results of these studies indicate that there are at least two different forms of X-linked DFN and argue for two separate MR genes on the proximal long arm of the X. Finally, deletion mapping, YAC screening and cosmid cloning has enabled us to construct a cosmid contig spanning (part of) the Norrie locus, and the search for expressed and evolutionarily conserved sequences is in progress.

Mutational Analysis

U 100 GENOTYPIC ANALYSIS OF ETHYLNITROSOUREA-INDUCED MUTATIONS BY TAQ I RFLP/PCR IN THE C-H-RAS PROTOONCOGENE, Susanna M. Chiocca, Martha S. Sandy and Peter A. Cerutti, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, 1066 Epalinges/Lausanne, Switzerland

In order to define the role of mutations in tumorigenesis higly sensitive technologies are required which allow their detection in disease-related genes in a few progenitor cells. In genotypic mutation analysis DNA sequence changes are determined without the in vivo or in vitro selection of phenotypically altered cells. We have studied the induction of base pair changes by N-ethyl-N-nitrosourea in TaqI endonuclease recognition site 2508-2511 (TCGA) of the c-H-ras1 gene in human fibroblasts by the RFLP/PCR method (RFLP, restriction fragment length polymorphism). Because this site contains all 4 base pairs it allows the detection of the 12 possible single base pair changes. The transition of G2510 to A2510 was the major mutation detected by λ-plaque oligonucleotide hybridization and quantitative sequence analysis of the RFLP/PCR products. It involves the G-residue of the CpG sequence of the coding strand. Data calibration with an internal mutant standard indicates that absolute frequencies for this transition lie in the range of $4\text{-}12 \times 10^{-7}$. The RFLP/PCR approach to genotypic mutation analysis holds promise for the measurement of early somatic mutations in disease-related genes in molecular epidemiology.

U 101 MOLECULAR DIAGNOSIS OF CYSTIC FIBROSIS (CF) IN MARITIME CANADA: A PROGRESS REPORT, DEC Cole, DC Riddell, PG Corsten, DC Hamilton, CE Blight and CT Gillespie, Depts of Pediatrics, Pathology and Statistics, Dalhousie University, Halifax, NS B3J 3G9

Many people in Maritime Canada trace their ancestry to some of the oldest settlements in North America. Both founder effect and genetic drift have been invoked to account for the anomalously high frequency of some disorders, such as PKU and Niemann-Pick D disease, and the virtual absence of others, notably fragile X. Although the birth prevalence rate for CF (1:2020, with correction for age-related ascertainment) is not different from other Canadian centers, we were concerned that different haplotype and ΔF508 frequencies might impair our ability to provide accurate counselling to at risk families. We collected samples from 64 of our 125 registered CF families, providing data on 103 non-CF and 118 CF chromosomes, as summarized below:

		CF	
Haplotype*	Normal	ΔF508**	Other
A	32	2	3
B	15	84	16
C	36	0	6
D	20	3	4

*Beaudet et al. (Am J Hum Genet 1989;44:319)
**75% of CF alleles

The standardized coefficient for linkage disequilibrium between XV2C and KM19 in non-CF chromosomes (0.04) was not significant ($p \neg 0.38$). The linkage disequilibrium between ΔF508 mutation, CF phenotype and RFLP haplotypes is marked, and not significantly different from that reported by Kerem et al. (Science 1989;245:1043). This was verified by tests for differences in association, based on log linear models (using GLIM routines). These data form the basis for calculating carrier risks for individuals currently undergoing haplotype and ΔF508 testing in our region.

Supported by the Canadian CF Foundation.

U 102 MUTANT β-HEX α- AND β- SUBUNIT GENES CAUSE INHERITED MOTOR NEURON DISEASE Glyn Dawson and Probal Banerjee, Univ. of Chicago, Depts. Peds. and BMB, Chicago, IL 60637.

Analysis of Hex A (αβ) and Hex B (ββ) enzymes in a patient (KL) with progressive motor neuron disease symptoms revealed partial presence of Hex A (20-50% of normal 4MUGlcNAc hydrolytic activity) and no Hex B. Purified KL Hex A showed reduced catalytic activity towards G_{M2}, but the levels of activator protein ($Hex_{activator}$) were normal in KL, suggesing that the defect was in the β- chains. Western blot analysis and separation of Hex A and Hex B proteins followed by immunoprecipitation showed that KL synthesized only 50% of the normal number of β-chains, and the β-chains thus synthesized associated only with α-chains and not with β-chains. Molecular analysis of the α-chains and the non self-associating β-chains derived from KL showed normal α- and β-chain mRNA but cloning and sequencing of PCR amplified cDNA revealed two different missense mutations (Types I and II) in the two β-chain alleles. The Type I mutation ($^{619}A \rightarrow G$) was paternally inherited and converted a $^{207}Ile \rightarrow Val$ in a highly conserved region believed to be associated with lysozyme-like catalytic activity. The Type II mutation ($^{1367}A \rightarrow C$) present in the other, maternally derived allele converted $^{456}Tyr \rightarrow Ser$ in a highly conserved carboxy terminal region which is believed to determine protein folding and subunit association. We therefore believe that the patient is a compound heterozygote. To dissect the individual effect of the two mutant alleles on the structure and activity of Hex A and B proteins, constructs were made by inserting restriction fragments containing the respective mutations from the patient (KL) into the corresponding, enzyme-deleted regions of the wild type β-chain cDNA ligated into the mammalian expression vector, pCMV6b. Transient expression of the β-chain in the absence and presence of α-chain (co-transfection) in COS-7 cells was achieved by the use of lipofectin and differential expression of β-Hex activity was observed. A second patient (CZ) with similar mild motor neuron disease symptoms was found to express less than 5% of the normal level of activity of either Hex A or Hex B as assayed with 4MU-β-GlcNAc (biochemically consistent with Juvenile Sandhoff disease). mRNA isolated from both fibroblasts and lymphocytes (transformed by E.B. virus) derived from patient CZ again showed normal size and amount of α- and β-chain message but Western blot analysis revealed degraded Hex A and B proteins. The derived cDNA was PCR-amplified and sequenced and the results will be compared to the mutations observed in KL and other patients with motor neuron disease and β-Hex deficiency. (Supported by USPHS Grant HD-06426 and MDA).

U 103 MUTATION CREATING A NEW SPLICE SITE IN THE GROWTH HORMONE RECEPTOR GENES OF 37 ECUADOREAN PATIENTS WITH LARON SYNDROME, Mary Anne Berg,*# Jaime Guevara-Aguirre,† Arlan L. Rosenbloom,‡ Ron G. Rosenfeld,+ and Uta Francke.*#+ *Howard Hughes Medical Institute, and Departments of #Genetics and +Pediatrics, Stanford University, Stanford, CA 94305; †Institute of Endocrinology, Metabolism, and Reproduction, Quito, Ecuador; ‡University of Florida, Gainesville, FL 32610

Laron syndrome (LS) is an autosomal recessive condition characterized by short stature, normal or elevated serum growth hormone levels, and resistance to growth hormone. We have studied LS patients from an inbred population in southern Ecuador (Rosenbloom, *et al., NEJM* 323(20):1367, 1990) in order to characterize the molecular defect(s) responsible for the disorder in this population. Southern blots of patient DNA probed with a human growth hormone receptor (GHR) cDNA (provided by W.I. Wood, Genentech) revealed no alteration in the restriction fragment patterns compared to controls. Denaturing gradient gel electrophoresis of PCR amplified segments, including all exons of the GHR gene from a LS obligate carrier, revealed a single polymorphism within the coding region. Sequencing revealed a nucleotide substitution which does not alter the encoded amino acid sequence directly, but creates a sequence within an exon that has considerable homology to the donor splice consensus sequence. We have shown that splicing of GHR mRNA in patients' lymphoblasts occurs virtually exclusively at this newly created donor splice site. The predicted GHR protein produced in these patients would have a deletion of 8 amino acids from the extracellular domain, including a potential N-glycosylation site that is conserved in all species for which the GHR amino acid sequence is known.

We developed an allele-specific oligonucleotide assay to detect the mutation rapidly and found that 37 of 38 Ecuadorean probands are homozygous for the mutation and all of 26 available parents were heterozygous, suggesting that a single mutant allele, identical by descent, is responsible for the majority of cases of LS in southern Ecuador. The substitution was not detected in 61 non-Ecuadorean subjects without LS.

We suspect that the predicted deletion of 8 amino acids from the growth hormone receptor protein disrupts function of the receptor, causing the LS phenotype. Carrier testing is possible in this population using the allele-specific oligonucleotide hybridization assay.

U 104 DETECTION OF FOUR CYSTIC FIBROSIS MUTATIONS WITH SOLID-PHASE MINISEQUENCING,

Anu Jalanko, Marjut Ranki and Hans Söderlund, Orion Corporation, Biotechnology, SF-00380 Helsinki, Finland

The solid-phase minisequencing method is designed to detect single base changes or small deletions or insertions in genes (Syvänen et al, Genomics 8:684, 1990). In this communication we describe the further development of this method for the detection of cystic fibrosis (CF) mutations ΔF508 (3 bp deletion), G542X (G->T), G551D (G->A) and R560T (G->C) either as independent tests or as a combined screening assay. In the solid-phase minisequencing method the DNA fragment of interest is biotinylated in one strand by amplification and immobilized onto a streptavidin-coated microtiter plate well. The mutation point is detected with a specific detection primer and a minisequencing reaction where only one labelled nucleotide is enzymatically incorporated. This method identifies the genotype of amplified leukocyte DNA specimens and also allows for quantitative measurements.

In the screening assay the fragments of CF gene exons 10 and 11 are amplified simultaneously. By choosing the appropriate DNA strands of each exon to be biotinylated by amplification, the screening can be done in two reactions. One reaction contains the four detection primers and only labelled dCTP and the other the primers plus labelled dATP, dTTP and dGTP. In the presence of a normal sequence, all four sites will incorporate only dCTP, whereas with a mutated allele one of the sites will incorporate dATP, dTTP or dGTP. The solid-phase minisequencing method is highly sensitive and specific and thus allows the use of radioactive labels with low specific activity, e.g. ^{3}H-labelled nucleotides, as well as hapten-labelled nucleotides with fluorogenic and luminogenic detection.

U 105 GENETIC MOUSE MODELS OF PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY, Jack P. Jenuth, Rupinder K. Toor, Ellen R. Mably and Floyd F. Snyder, Departments of Pediatrics and Medical Biochemistry, University of Calgary, Calgary, Alberta T2N 4N1 Canada

We have recovered five independent mutations at the PNP locus in the progeny of ethylnitrosourea treated male mice mated to untreated females (Genome 32, 1026, 1989). Two of these mutations have been studied at the 6th generation backcross to C57BL/6J (B6). The B6-NPE and B6-NPF homozygous mutants have 4.4 and 1.1% of B6 PNP activity in erythrocytes. NP-1E determines a more basic protein on IEF gels than parental NP-1D, and NP-1F is unchanged. Michaelis constants are not altered for either mutation. Mutant mice excrete nucleosides in proportion to the severity of the enzyme deficiency at 10- and 100-fold greater than control levels. There was no evidence for dGTP accumulation in erythrocytes, thymocytes or spleen leukocytes of mutant mice. A secondary deficiency of deoxyguanosine kinase appears to account for the absence of dGTP accumulation with NP-1E and NP-1F having 22 and 14% of NP-1D activity respectively. Examination of thymocytes revealed a 10-fold increase to 40% of the total cells being double negative CD4^{-}CD8^{-} prothymocytes in the more severe B6-NPF mutants. We have cloned and sequenced the background mouse PNP cDNA allele, NP-1^{d} (Nuc. Acid Res. 19, 1708, 1991). The coding portion of the mutant genes was amplified and sequenced, revealing single base substitutions. B6-NPE is characterized by an ATG to AAG substitution at base 345 corresponding to a change in methionine 87 to lysine, consistent with the basic pI shift. B6-NPF has a single substitution at base 758, GCA to ACA, corresponding to a change in alanine 228 to threonine. We believe this to be valuable genetic model in which to explore the metabolic and physiological response to various therapeutic strategies.
(Supported by MRC of Canada, grant MT-6376.)

U 106 *IN VIVO* MISLOCALIZATION OF CFTR IN CYSTIC FIBROSIS SWEAT DUCT, Norbert Kartner and John R. Riordan, Departments of Biochemistry and Clinical Biochemistry, The Hospital for Sick Children and The University of Toronto, Toronto, M5G 1X8, Canada.

The product (CFTR) of the gene affected in cystic fibrosis (CF) is a low-conductance (5-10 pS), linear, cAMP-regulated chloride channel, usually expressed in apical membranes of polarized epithelia in some exocrine tissues. We have developed a panel of monoclonal antibodies directed towards the R domain and first and second nucleotide binding folds of CFTR to probe its structure and function. Western blotting of cultured epithelial cell membranes using these antibodies identifies a 170 kDa band with an intensity that agrees with the level of CFTR mRNA expression. Confirmation of the specificity of the antibodies is seen in Western blots of membranes from CFTR transfectants in mouse fibroblasts and CHO cells, and in recombinant baculovirus-infected Sf9 insect cells (where a minimally glycosylated 140 kDa band is observed). The detection of the putative CFTR band also correlates with the appearance of a 5-10 pS, linear, chloride-selective anion channel. By immunohistochemistry, CFTR has been localized to normal apical membranes of pancreatic intercalated duct, salivary gland striated duct, gut crypt cells, and apical and basolateral membranes of sweat duct. Staining is also seen in plasma membranes of developing spermatids. Unexpectedly, little staining is seen in airway epithelia, indicating a level of expression below our present limit of detection. These observations are in good agreement with *in situ* hybridization localization of CFTR (Trezise and Buchwald, *Nature 353:* 434, 1991). By contrast, in sweat ducts from CF patient skin biopsies, the antigen does not appear at the apical membrane, but rather as perinuclear aggregates, suggesting retention and degradation in the rER as a mechanism of chloride channel failure in CF. These results appear to confirm *in vivo*, in CF patient tissue, the previous observations of Cheng et al. (*Cell 63:* 827, 1990) that were made *in vitro* in heterologous expression systems, and to support their speculation that the ΔF508 missense mutation results in arrested transport of CFTR to the plasma membrane. We will conduct further studies on skin biopsies from CF patients other than ΔF508 homozygotes in an attempt to correlate CFTR localization with severity of disease (Supported by the Canadian and U.S. CF Foundations and the NIH-NIDDK).

U 107 IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS IN *mut*$^{-}$ METHYLMALONIC ACIDEMIA, Nandini Kogekar and Wayne A. Fenton, Dept. of Genetics, Yale Univ. School of Medicine, New Haven CT 06510

The *mut* class of methylmalonic acidemia, an inborn error of amino acid and organic acid metabolism, is caused by inherited deficiency of methylmalonyl-CoA mutase (MUT). Two sub-classes have been defined in cultured fibroblasts from patients: *mut*^{0}, with no residual activity, and *mut*$^{-}$, with some residual activity showing a very elevated K_m for the cofactor, adenosylcobalamin. We have sequenced MUT cDNA from three *mut*$^{-}$ patients directly after reverse transcription of RNA, obtained from fibroblast cells, and PCR amplification. Five different missense sequence changes have been identified. Cell line 394 showed a homozygous $T^{787}\rightarrow A$ ($Tyr^{231}\rightarrow Asn$). Cell line 378 showed two heterozygous changes: $G^{1182}\rightarrow A$ ($Arg^{368}\rightarrow His$), and $A^{1671}\rightarrow G$ ($His^{532}\rightarrow Arg$). Cell line 515 showed three changes: homozygous $A^{1671}\rightarrow G$ ($His^{532}\rightarrow Arg$), and heterozygous $G^{357}\rightarrow T$ ($Gly^{84}\rightarrow Val$) and $G^{2087}\rightarrow A$ ($Val^{671}\rightarrow Ile$). Two of these changes, $A^{1671}\rightarrow G$ and $G^{2087}\rightarrow A$, have been found in other *mut* cell lines and have been reported to have no effect on MUT activity upon expression of these variant proteins in mammalian cultured cells. Variant enzymes containing each of the other changes are being expressed in a prokaryotic system to determine which are responsible for the biochemical phenotype of these cell lines. Their kinetic parameters and thermostability will be compared with those determined previously for the residual enzyme present in crude extracts of these patient fibroblast lines.

U 108 DGGE ANALYSIS OF MUTATIONS CAUSING GAUCHER'S DISEASE, K. H. Laubscher[1], V. Gopalan[2], R. H. Glew[2], R. T. Okinaka[1], [1]Los Alamos National Laboratory, Los Alamos, NM 87545 and [2]University of New Mexico, Albuquerque, NM 87131. Deficiencies in human beta-glucocerebrosidase activity results in various forms of the lysosomal disorder known as Gaucher's disease. Current investigations are attempting to correlate molecular changes in the gene that encodes for this protein with the clinical manifestations of the disease. Since the majority of mutations identified in Gaucher's patients occur in exons 9 and 10, complete analysis of these two exons will reveal most patient mutations. We are utilizing a denaturing gradient gel electrophoresis (DGGE) technique to rapidly analyze mutations in exon 10 of the glucocerebrosidase gene. PCR primers were designed to preferentially amplify exon 10 while excluding the 96% homologous sequences from the pseudogene. The first round of PCR took advantage of pseudogene deletions to amplify only active gene sequences. The second round of nested PCR utilized primers immediately outside exon 10, with one primer containing a 42 bp GC-clamp. A 0-100% (7M urea, 50% formamide = 100%) denaturing gradient gel allowed rapid analysis of the 117bp exon 10 fragment. DGGE readily distinguished sequence differences between wildtype and mutant DNAs. DGGE analysis also revealed recombinants between the active gene and pseudogene. Coupled with direct-sequencing strategies DGGE analysis revealed the prevalent T to C transition at base pair 6433 (1448 in the cDNA) and two recombinant genotypes containing all three pseudogene base pair substitutions. DGGE analysis of exon 9 is currently under investigation to enable complete screening of exons containing the majority of mutations. This research funded by DOE, and Los Alamos LDRD funds.

U 109 DIFFERENTIAL TISSUE-SPECIFIC PATTERN OF TRANSCRIPTION OF THE NADH-CYTOCHROME b5 REDUCTASE GENE INVOLVED IN CONGENITAL METHEMOGLOBINEMIAS, Alena Leroux, Luisa Mota Vieira, Jean-Claude Kaplan and Axel Kahn, Institut Cochin de Génétique Moléculaire (ICGM), INSERM U129, 24 rue du Faubourg Saint Jacques, 75014 Paris, France

There are two types of recessive congenital methemoglobinemia [Mc Kusick N° 25800] due to a same defective gene : NADH-cytochrome b5 reductase (cytb5r) (DIA1 locus 22q13.31-qter). In type I disease, cyanosis is the only symptom, and the enzyme is only defective in RBC ; in type II disease, cyanosis is associated with severe mental retardation and neurological impairment, and the enzyme defect is systemic.

Two cytb5r isoforms have been described: a soluble protein predominant in RBC, and a membrane-bound protein located in endoplasmic reticulum of all tissues. Both of them are derived from a single gene and it was currently believed that the soluble form was generated by proteolytic cleavage of an N-terminal hydrophobic peptide.

In the present study we report on the existence of three different species of cytb5r mRNAs detected in rat liver and reticulocytes using the anchored PCR technique. The first exon of liver cytb5r mRNA is identical to that already published, while two other cytb5r mRNAs found in rat reticulocytes share the common second exon with liver mRNA, but are preceded by two different sequences at their 5' end.

According to these results, the existence of three promotors within the cytb5r gene should be considered : the first one seems to be ubiquitously active; the second one is preferentially used in erythroid cells; the third one is ubiquitous, but very weak.

The identification of different cytb5r mRNA species, differentially expressed in tissues, should help understanding the mechanism by which two phenotypically different diseases are produced by allelic defects of a single gene. The molecular pathology of this gene in patients affected with either type I or type II disease is currently being investigated.

U 110 GENOTYPE - PHENOTYPE RELATIONSHIPS AND GENE INTERACTIONS IN β-THALASSEMIA, Ariella Oppenheim[1], Dvorah Filon[1], Tina C. Warren[2], Eliezer A. Rachmilewitz[1], Haig H. Kazazian[2], Jr. and Deborah Rund[1], [1]Dept. of Hematology, Hadassah Univ. Hospital, Jerusalem, Israel 91120; [2]Medical Genetics Center, Johns Hopkins Hospital, Baltimore, Md 21205

In Israel, β-thalassemia is widespread, in particular among certain ethnic groups. Mutational analysis has so far revealed 22 different mutations, 6 of which are unique to Israel. This remarkable heterogeneity affords detailed studies on genotype-phenotype relationships. The results indicate that certain mild mutations underlie thalassemia intermedia in homozygotes. Some of these are associated with silent carriership. Most surprisingly, in heterozygotes we found a strong correlation between the type of the mutation and the degree of microcytosis (reduction in the red blood cell volume, MCV). All the mutations leading to a β^0 (null) phenotype were associated with a lower MCV than those of β^+ phenotype. Moreover, the MCV values for each of the various β^+ mutations were clustered, and the mean MCV of the cluster correlated with the severity of the mutation. These direct genotype-phenotype relationships suggest the intriguing possibility that the level of β-chains, or excess α-chains, determines the size of the mature erythrocyte. Studies of interactions of β-thalassemia with other genetic determinants reveal multiple mechanisms ameliorating the phenotype. These include mutations in γ-globin, α-globin and other unlinked genetic factors.

U 111 GERMLINE MUTATIONS WITHIN THE WILMS' TUMOR GENE, WT1, ARE PRESENT IN INDIVIDUALS WITH DENYS-DRASH SYNDROME. Jerry Pelletier, Wendy Bruening, and David Housman.McGill Cancer Center, McGill University, Montreal, Canada, H3G 1Y6 and M.I.T. Cancer Center, Boston, MA 02139.

Denys-Drash syndrome is a rare human condition in which severe aberrations of the urogenital system result in renal failure and pseudohermaphroditism. The nephropathy is generally characterized by a specific glomerular lesion - accumulation of matrix material in the mesangium, surrounded by hypertrophied epithelial cells. Wilms' tumor (WT) is also a cardinal feature of this syndrome. Denys-Drash individuals usually have bilateral WT and present earlier than individuals with sporadic tumors, suggesting the presence of a predisposing germline mutation in these individuals.

We have have identified germline mutations within the DNA-binding, zinc-finger domains of the Wilms'tumor suppressor gene,WT1, in ten individuals affected with this disease. In three families analyzed, the mutations arose *de novo*. These alterations affect amino acids predicted to participate in hydrogen bonding with a guanine residue in the major groove of the WT1 binding site. We show that these mutations directly affect DNA sequence recognition. Retrospective analysis on three Wilms' tumor and a juvenile granulosa cell tumor from three Denys-Drash individuals demonstrated reduction to homozygosity for the mutated WT1 allele. Our results are discussed in light of recent evidence implicating *WT1 in genital system development.*

U 112 **SPECTRUM OF MUTATIONS CAUSING ASPARTYLGLUCOSAMINURIA, A LYSOSOMAL ACCUMULATION DISEASE**, L. Peltonen[1], E. Ikonen[1], P. Aula[2], O.K. Tollersrud[1] and A.-C. Syvänen[1], [1]Laboratory of Molecular Genetics, National Public Health Institute, Helsinki; [2]Department of Medical Genetics, University of Turku, Turku, Finland

Aspartylglucosaminuria (AGU) is a lysosomal accumulation disease caused by the deficiency of aspartylglucosaminidase (AGA), the enzyme hydrolyzing the Asn – N-acetylglucosamine linkage as the final step in the degradation of glycoproteins. We have earlier reported cloning of a 2.1 kb AGA cDNA encoding the 346 aa long AGA polypeptide. We also found that a single missense mutation AGU_{Fin} (Cys_{163} -> Ser) causes the disease in Finland, the population demonstrating the majority of AGU cases worldwide (1). Here we describe the spectrum of 12 AGU mutations in unrelated patients from diverse ethnic backgrounds. All Scandinavian patients had AGU_{Fin} whereas the patients outside Scandinavia all carried different mutations and majority of them (11/12) were homozygotes for a given mutation. The mutations, representing except point mutations also deletions, insertions and splicing defects are distributed over the entire coding region of the gene (2). Like AGU_{Fin} also most other mutations affect the folding and stability and not directly the active site of the enzyme (3). Currently in vitro mutagenesis of selected amino acids and consequent expression in COS cells combined with chemical modifiers are used to analyze the amino acids important in the formation of the active center of this lysosomal enzyme.

References: Ikonen et al 1) The EMBO J 10:51-58, 1991; 2) Proc Natl Acad Sci USA in press 3) Genomics 11:206-211, 1991;

Grants: The Academy of Finland, Sigrid Juselius Foundation

U 113 **MOLECULAR PATHOBIOLOGY OF GALACTOSEMIA: MUTATIONS AND POLYMORPHISMS IN HUMAN GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE**, Juergen K.V. Reichardt and Savio L.C. Woo, Howard Hughes Medical Institute and Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Classic galactosemia (McKusick 230400) is caused by deficiency of the ubiquitously expressed enzyme galactose-1-phosphate uridyl transferase (GALT; EC 2.7.7.12). Acute symptoms include vomiting, failure to thrive, hepatosplenomegaly and E.coli sepsis. These complications can be avoided by placing afflicted individuals on a galactose-restricted diet. However, late-onset symptoms, including neurologic abnormalities and ovarian failure, persist.

We have characterized by PCR-amplification, direct sequencing, population screening and expression analysis eight galactosemia mutations and three GALT polymorphisms. Eleven nucleotide changes resulted in missense mutations while one was a silent third base pair substitition. Seven of the eight galactosemia mutations resulted in stable immunoreactive protein with little or no residual activity. These mutations affected evolutionarily conserved domains of GALT. The three polymorphisms, in contrast, resulted in proteins with near normal specific activities and were found in non-conserved areas of the enzyme. Seven of the eight galactosemia mutations and all three polymorphisms were rare in our patient population. Only one mutation, Q188R, was common, accounting for about 1/3 of our mutant alleles. The molecular heterogeneity we have observed may explain the variable clinical outcome typical of galactosemia patients. Finally, each of the seven patients we have fully genotyped bears at least one galactosemia allele predicted to have residual activity. This finding may be important in understanding the biochemical basis of the chronic symptoms and in addressing the long-term complications of this disease.

U 114 HIGHLY SENSITIVE NON-RADIOACTIVE LABELING AND DIDEOXY SEQUENCING BASED ON THE DIGOXIGENIN (DIG) SYSTEM, R. Rüger, G. Sagner, H.-J. Höltke, R. Seibl, U. Reischl and C. Kessler, Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg, FRG

Non-radioactive labeling and detection methods are of increasing importance in diagnosis and research. A system based on hapten digoxigenin (DIG) and anti-hapten (anti-DIG) enzyme linked immunosorbent assay (ELISA) was developed.

The DIG-system allows the highly sensitive and specific detection of nucleic acids (≤ 0.1 pg or single copy genes in genomic DNA) in the dot-, slot-or blot-formats both with colorimetric substrates (BCIP/NBT) or with the chemilumi-nescent substrate DPP ($AMPPD^R$/Tropix, Inc. or $Lumi\text{-}Phos^R$/Lumi Gen, Inc.).

DIG-[11]-2'-desoxyuridin-5'-triphosphate (Dig-[11]-dUTP) is accepted by the Thermus aquaticus (Taq) DNA polymerase during polymerase chain reaction (PCR). DIG-labeled PCR products can either be detected after gel electrophoresis and Southern transfer at a sensitivity of 5-25 attogram starting concentration of specific sequences or can be used as vector-free highly sensitive hybridization probes.

The DIG-system is also easily applicable for the enzymatic dideoxy sequencing technique. Combined with a simple and efficient blotting procedure from standard sequencing gels or with a direct blotting electrophoresis (DBE) device the system yields clear and background free sequence data. The resolution of the sequence ladder is analogous to radioactive sequencing and using the chemiluminescent substrate DPP the detection time can be reduced to 10-60 min.

The described applications of the DIG-system combine the advantages of non-radioactive nucleic acid labeling with high sensitivity, specificity and rapidity for the detection of nucleic acid sequences.

U 115 **QUANTITATIVE DETERMINATION OF MUTANT ALLELES AND THEIR TRANSCRIPTS BY PCR AND SOLID-PHASE MINISEQUENCING**, Syvänen, A-C.[1], Ikonen, E.[1], Manninen, T.[1], Söderlund, H.[2], Palotie A[3]., Peltonen, L.[2], [1]Laboratory of Molecular Genetics, National Public Health Institute, Helsinki, Finland; [2]Orion Corp., Biotechnology, Helsinki, Finland; [3]Department of Clinical Chemistry, University of Helsinki, Finland.

Studies on human genetic diseases often require quantitative analysis of minute amounts of DNA or RNA. We present a new method, in which the limitations of current quantitative PCR methods can be overcome. In this method two DNA sequences differing from each other by a single nucleotide are co-amplified using a biotinylated PCR primer. The biotinylated PCR product is immobilized on a streptavidin coated solid support and denatured. The ratio between the two sequences in the PCR product is determined by separate primer extension reactions, in which a detection step primer annealing immediately adjacent to the site of the variable nucleotide is elongated by a DNA polymerase with a single labelled dNTP complementary to the nucleotide at the variable site. The ratio between the labels incorporated in this reaction accurately determines the ratio between the two sequences in the original sample without any gel electrophoretic separation step. The method is highly sensitive allowing quantification of a mutant sequence present in less than 1% of a sample. We have applied this method to determine the carrier frequency of a Finnish recessive disease, aspartylglucosaminuria, by quantifying the amount of mutant allele present in a pooled leukocyte sample from over 1000 normal individuals. In another application we were able to accurately determine the proportion of blast cells carrying mutations in the N-ras gene in patients with acute myeloid leukemia. The solid-phase minisequencing technique is generally applicable for quantification of any DNA or RNA in a sample after addition of a known amount of standard nucleic acid differing from that to be quantified by one nucleotide. For analysis of RNA, an RNA standard is preferably added before the first strand cDNA synthesis step preceding PCR.

U 116 FUNCTIONAL SUBSTITUTION OF THE FIRST NUCLEOTIDE BINDING FOLD OF THE STE6 GENE OF YEAST WITH CFTR. John Teem and Lap-Chee Tsui. Department of Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.
The cystic fibrosis transmembrane conductance regulator gene (CFTR) encodes an integral membrane protein having structural similarity to the yeast STE6 gene, transporter of the mating pheromone a-factor. Most of the similarity between these genes occurs in the first nucleotide binding fold, which in CFTR is the location of the major mutation resulting in cystic fibrosis, a deletion of the phenylalanine codon at amino acid position 508 (ΔF508). In order to determine whether the nucleotide binding fold of CFTR and STE6 are functionally similar, a series of substitutions of the CFTR NBF1 were made in the STE6 gene. One particularly interesting STE6/CFTR hybrid gene was constructed in which 60% of the CFTR nucleotide binding fold (including F508) replaced the corresponding region of the STE6 gene. The hybrid gene could complement a ste6 deletion mutation, allowing the yeast cells to export a-factor and mate. More important, the mating efficiency was drastically reduced when the ΔF508 mutation was introduced into the hybrid gene. These results suggest that the nucleotide binding fold of CFTR and STE6 share a similar functional conformation and that the Δ508 mutation likely alters this conformation, possibly interfering with ATP hydrolysis. Further, the mating assay provides a convenient means to isolate mutations in the hybrid gene that restore the conformation and function to the Δ508 mutant CFTR nucleotide binding fold.

Cancer Genetics and Tumor Suppressor Genes

U 200 PHYSICAL MAPPING OF THE t(2;13) TRANSLOCATION BREAKPOINT IN ALVEOLAR RHABDOMYOSARCOMA, Frederic G. Barr[1,2], John Holick[1], Lynn Nycum[1], Jaclyn Biegel[1], and Beverly S. Emanuel[1], [1] Division of Human Genetics and Molecular Biology, Children's Hospital of Philadelphia and [2] Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104.
A characteristic translocation t(2;13)(q35;q14) has been previously identified in the pediatric soft tissue tumor alveolar rhabdomyosarcoma. To localize the translocation breakpoint on physical maps of the distal 2q and proximal 13q regions, we have developed a two step mapping strategy. We first assembled panels of lymphoblast, fibroblast, and somatic cell hybrid cell lines with deletions and unbalanced translocations involving chromosomes 2 and 13. Southern blot analysis of these mapping panels with probes from chromosomes 2 and 13 facilitated assignment of the corresponding loci to defined physical intervals. The position of these probes with respect to the t(2;13) rhabdomyosarcoma breakpoint was then determined by either quantitative Southern blot analysis of an alveolar rhabdomyosarcoma cell line with two copies of the derivative chromosome 13 and one copy of the derivative chromosome 2 or PCR analysis of somatic cell hybrids derived from an alveolar rhabdomyosarcoma cell line. Using these methodologies, we have identified the physical mapping intervals which contain the t(2;13) breakpoints; this information will permit rapid determination of the proximity of new cloned sequences to the translocation breakpoint. Furthermore, from a comparison of our data with the human and syntenic mouse linkage maps, we have identified loci which closely flank the translocation breakpoint; these loci will serve as starting points for subsequent chromosomal walking approaches for cloning the regions disrupted by the t(2;13) translocation.

U 201 THE t(15;17) TRANSLOCATION OF ACUTE PROMYELOCYTIC LEUKEMIA GENERATES A FUNCTIONALLY ALTERED RETINOIC ACID RECEPTOR. Anne Dejean, Catherine Lavau, Christine Chomienne*, Agnès Marchio, Laurent Degos* and Hugues de Thé, UREG, Institut Pasteur and *Hôpital Saint-Louis, Paris, France.
A specific translocation t(15,17) has been reported in every patient with acute promyelocytic leukemia (APL). We have previously reported that, in an APL-derived cell-line (NB4), this translocation fused the retinoic acid receptor α (RARα) gene to a previously unknown locus, initially called *myl* and now renamed PML. Moreover, genomic alterations of either RARα or PML loci were demonstrated in most patients, suggesting that the fusion of these two genes might be a general situation in APL.
We now report the molecular cloning of the wild-type PML and hybrid PML-RARα transcripts. The PML gene product displays a C_3HC_4 motif found in several DNA-binding proteins and could encode a transcription factor. Two hybrid cDNAs, that differ by an alternatively spliced coding exon of PML, were isolated from the NB4 cell-line and shown to encode proteins containing most of the PML sequences fused to a large part of RARα, including its DNA- and hormone- binding domains. In transient expression assays, the hybrid protein exhibits altered *trans*-activating properties if compared to the wild-type RARα progenitor. These observations suggest that in APL, the t(15;17) translocation generates a retinoic acid receptor mutant that could contribute to leukaemogenesis through interference with promyelocytic differentiation. The analysis of the transforming properties of the PML-RARα hybrid are in progress.

U 202 A NOVEL APPROACH TO GENETIC DISSECTION OF CANCER AND OTHER MULTIFACTORIAL DISEASES IN THE MOUSE : MAPPING OF FOUR NEW COLON CANCER SUSCEPTIBILITY GENES. P. Demant, C.J.A. Moen, P.C. Groot, M. Snoek, M.A. van der Valk, L.F.M. van Zutphen, W. Dietrich, E.S. Lander, and O. von Deimling. The Netherlands Cancer Institute, 1066CX Amsterdam, and Dept. Animal Science, State University Utrecht, The Netherlands, The Whitehead Institute, Boston, Mass., U.S.A., and Dept. Biochemical Pathology, Albert-Ludwigs University, Freiburg, Germany.

The understanding of the genetic control of susceptibility to cancer and a number of other diseases has been hampered because their development is determined by multiple loci. To overcome this obstacle we developed a new genetic tool, the Recombinant Congenic Strains (RCS). A series of RCS comprises about 20 inbred strains, each of which contains approx. 88% genes from one inbred strain - the "background" strain, and approx. 12 % genes from a second inbred strain - the "donor" strain. As the subset of the donor strain's genes is different in each RC strain, the individual genes of the donor strain involved in the control of a multigenic trait become segregated into different RC strains, where they can be mapped and analyzed separately.

We applied the RCS system to the study of colon cancer in mice. The RC strains of the BALB/c-c-STS (CcS/Dem) RCS series, with the background strain BALB/c (resistant to colon tumors) and donor strain STS (highly susceptible) differ widely in colon cancer development after DMH treatment. Backcross mice of several highly susceptible RCS to BALB/c were tested for colon tumor development and typed for genetic markers of the STS strain. 4 novel non-linked loci, Scc-1 (Susceptibility to colon cancer-1) through Scc-4, were mapped to chromosomes 2, 7, 11, and 10, respectively. These Scc loci are different from the genes which are frequently altered in colon cancer cells (ras, p53, MCC, DCC, and APC/FAP).

This result opens new avenues for study of genetic determination of colon cancer and indicates that the RCS system can be used to dissect the complex genetics of cancer and other diseases.

U 203 YEAST ARTIFICIAL CHROMOSOME (YAC) CLONING OF THE t(4;11) ACUTE LYMPHOBLASTIC LEUKEMIA BREAKPOINT, Peter H. Domer, Gary A. Silverman, Dan Chen, John H. Kersey, Stanley J. Korsmeyer, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110, University Minnesota Medical Center, Minneapolis, MN 55455

The t(4;11)(q21;q23) is a characteristic breakpoint in a $CD34^{+}CD19^{+}CD10^{-}CD15^{+}$ pre B/monocytic acute lymphoblastic leukemia (ALL). The breakpoint is distant from known molecular markers. We approached this translocation by obtaining YAC clones of genes mapping to 11q23 from the Washington University YAC library. yB22B2, a 280 kb YAC containing CD3δ/γ spanned the 11q23 breakpoint as determined using somatic cell hybrids containing the der(4) and der(11) chromosomes of the RS4;11 cell line. We constructed a detailed restriction map and λ phage contig of subclones of this YAC. The breakpoint of RS4;11 and several 11q23 ALLs were identified within a limited breakpoint region telomeric to CD3γ/δ. The RS4;11 breakpoint has been cloned and sequenced demonstrating the translocation has occurred in a region of repetitive sequence DNA. An evolutionarily conserved sequence of 11q23 origin recognized a cDNA from a RS4;11 cDNA library. This cDNA identifies a transcriptional unit of over 10 kb which spans the breakpoint region. Thus, YAC cloning has identified a chromosome breakpoint region distant from known genes and revealed a transcriptional unit from the breakpoint region of a poor prognosis ALL.

U 204 INVOLVEMENT OF TUMOR SUPPRESSOR GENES IN HUMAN RENAL CELL CARCINOMA, James Gnarra, Farida Latif, Kalmon Tory, Patrick Anglard, Robert Reiter, John Long, Emile Trahan, Sue Liu, Michael Lerman, Berton Zbar, and W. Marston Linehan, Surgery Branch and Laboratory of Immunobiology, NCI, NIH, Bethesda, MD 20892.

Consistent abnormalities on the short arm of chromosome 3 are seen in human renal cell carcinomas (RCC). Deletions causing loss of chromosome 3p heterozygosity (LOH) in tumors from 88% (51 of 58) evaluable patients was previously demonstrated. In addition, when tumor-derived RCC cell lines were examined, 89% (25 of 28) showed LOH at chromosome 3p. Such a high frequency of chromosome 3p deletions supports the hypothesis that a tumor suppressor gene(s) at that locus may be central to RCC development. Restriction fragment length polymorphism analyses of RCC cell lines at loci containing other well characterized tumor suppressor genes showed lower frequencies of LOH: 45% LOH at 13q, the retinoblastoma locus; 48% LOH at the p53 locus and 18% at the nm23 locus, both at 17q; 40% LOH at 18p, the DCC locus. These results imply that these tumor suppressor genes may not play a role in initiation of RCC, but their importance for disease progression has yet to be ascertained. Based on RFLP analyses of RCC-derived cell lines the minimal chromosome 3p deletion involved in sporadic RCC spans bands 3p14 to 3p26. This region contains the von Hipple-Lindau (VHL) disease locus which was mapped to 3p25-26. A molecular genetic approach was undertaken to identify the gene(s) responsible for sporadic and familial RCC. Molecular probes from a human chromosome 3-specific cosmid library were mapped within the VHL disease gene region. These probes were used to initiate cosmid and yeast artificial chromosome walking in order to isolate the surrounding region of chromosome 3p. In addition, evolutionarily conserved cosmid inserts were used to screen human kidney cDNA libraries. Several independent cDNA clones have been identifiedand their DNA sequences are being determined. Tissue distribution of mRNA expression is also being evaluated. These cDNA clones, therefore, represent candidate genes which may be responsible for VHL disease and sporadic RCC.

U 205 SEQUENCE AND ANALYSIS OF THE THROMBOSPONDIN PROMOTER AND ITS REGULATION BY A TUMOR SUPPRESSOR GENE. Deborah J. Good, Jianxun Gui, Paul Framson, Paul Bornstein and Noel P. Bouck, Department of Microbiology/Immunology and Cancer Center, Northwestern University, Chicago, Illinois 60611, and Department of Biochemistry, University of Washington, Seattle, Washington 98195

Baby hamster kidney (BHK) cells transform from anchorage dependent and non-tumorigenic to anchorage independent and tumorigenic in a single step as a result of the inactivation of a tumor suppressor gene. One function of this suppressor gene is to control the elaboration of a matrix glycoprotein able to inhibit neovascularization *in vivo*. This inhibitory protein is a 140 kD isoform of thrombospondin-1(TSP-1). Normal, non-angiogenic BHK cells containing an active suppressor gene make 4-6 fold more gp140 protein than transformed angiogenic cells lacking this suppressor gene. This increased amount of protein is not due to increased stability of gp140 in the suppressed cells or their conditioned media, as pulse chase immunoprecipatation experiments indicated that the half-life of the inhibitory protein is approximately the same for both cell types. Regulation of gp140 is at the RNA level. When BHK cells containing a temperature sensitive tumor suppressor gene were grown at 38C, where the tumor suppressor gene was inactive, the amount of TSP RNA was low. Conversely, at 32C where the tumor suppressor gene was active, the amount of TSP RNA returned to levels comparable to those of normal BHK cells. Using actinomycin D to inhibit new mRNA synthesis, the stability of TSP mRNA was found to be similar in the presence and absence of a suppressor gene. Southern analysis showed that a single gene in BHK cells encodes the hamster gp140 protein. A hamster genomic library (λDASH, Stratagene) was screened using a human TSP cDNA probe (kindly provided by Dr. William Frazier, St. Louis) and a 14-kbp clone containing approximately 6-kbp of the hamster TSP promoter as well as the majority of the protein coding region was isolated. Sequence analysis of a 4.2-kbp fragment containing 0.6-kbp of promoter and 3.6-kbp of coding regions indicates that the hamster TSP gene is very similar to the human gene, THBS-1. Transcription of both genes begins approximately 23 bp after the TATA box, as determined by primer extension analysis. Deletional analysis of the human TSP promoter driving a chloramphenicol acetyl transferase gene indicates that only a minimal promoter region along with the first exon and intron sequences (-38 - +750) are sufficient to maintain suppressor gene control. Further analysis is underway to determine exactly how the tumor suppressor gene in BHK cells depresses angiogenesis via its positive regulation of thrombospondin.

U 206 ANALYSIS OF A TRANSLOCATION BREAKPOINT IN A SPORADIC MENINGIOMA AND A CONSTITUTIONAL ABERRATION ON CHROMOSOME 22 IN A PUTATIVE NF2 PATIENT, Ronald H. Lekanne Deprez, Nicole A. Groen, Peter H.J. Riegman, Ursula L. Kortenhorst and Ellen C. Zwarthoff, Department of Pathology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands.

Neurofibromatosis 2 (NF2) is an inherited disorder in which acoustic neuromas, meningiomas and other central nervous system tumors occur. The gene predisposing to NF2 has been mapped to chromosome 22. In addition loss of chromosome 22 has been observed in NF2 associated tumors and in their sporadic variants. This suggests that a tumor suppressor gene on chromosome 22 is involved in the pathology of these tumors. In a meningioma from one particular patient we detected a reciprocal t(4;22)(p16;q11). The translocation breakpoint presumably disrupts the tumor suppressor gene. Using rare cutting restriction enzymes and CHEF electrophoresis we found that probe NB129 probably detects the translocation breakpoint. In addition this probe detects a small constitutional deletion in an NF2 patient (patient 55). In a meningioma from this patient the chromosome containing the deletion is present whereas the other copy of chromosome 22 is lost. Using probes from an area surrounding the deletion we have isolated a number of cDNA clones which are being analysed at present.

U 207 EXON AMPLIFICATION OF A CHROMOSOME 21 SPECIFIC YAC SPANNING THE t(8;21) BREAKPOINT OF ACUTE MYELOGENOUS LEUKEMIA, Paul Nisson [1], Paul Watkins [1], Alice Young [1], Lyndal Kearney [2], Bryan Young [2], and Nicoletta Sacchi [3]

[1] Life Technologies, Inc., Gaithersburg, MD; [2] Imperial Cancer Research Fund, London; [3] School of Medicine, University of Milan

Analysis of non-random genome rearrangements associated with specific hematological neoplasms has shown the deregulation or disruption of a number of genes at the breakpoint regions. The mechanism of action of most of these genes is related to the signal transduction cascade (1). Positional cloning has allowed us to isolate a 240 kb human genomic fragment specific for the chromosome 21 locus D21S65 in a yeast artificial chromosome (C4C10 YAC). In situ hybridization experiments showed that this YAC identifies the t(8;21)(q22;q22) breakpoints in metaphases of blasts of patients with acute myelogenous leukemia. Suppression hybridization of the C4C10 YAC DNA to human, mouse, and hamster genomic DNAs clearly identifies DNA sequences in the three species, strongly suggesting that in the 240 kb fragment there may be highly conserved transcribed genes (2). To isolate the expressed sequences in the YAC we used an exon amplification strategy based on RNA splicing previously used for the isolation of expressed sequences from cosmid and phage DNA clones (3). PCR analysis of RNA preparations from Cos cells transfected with shotgun subclones of the C4C10 YAC in the splicing vector pSPL1 generated multiple products representing putative trapped exons. Sequence analysis of these clones is expected to identify one or more genes at or near the translocation breakpoint on chromosome 21.

(1) Solomon E. et al. Science 254:1153-59, 1991.
(2) Kearney L. et al. Cancer Genet Cytogenet 57:109-19, 1991.
(3) Buckler A.J. et al. Proc Natl Acad Sci (USA) 88:4405-9, 1991.

U 208 REVERSAL OF THE MALIGNANT PHENOTYPE OF CULTURED MELANOMA CELLS VIA DNA TRANSFECTION: POTENTIAL FOR DIRECT SELECTION AND CLONING OF TUMOR SUPPRESSOR GENES. John F. Stone, Southwest Biomedical Research Institute, Scottsdale, AZ

Microcell transfer of normal chromosomes into cultured cancer cells has shown that the malignant phenotype of the cells can be reversed, presumably by the introduction of tumor suppressor genes encoded on the introduced chromosome. A major drawback to this approach is that the suppressor genes cannot be selected for directly. As an alternative, we have utilized the selective killing of malignant cells by Rhodamine 123 (R123) following DNA transfection to select for cells in which tumor suppression has occurred. Cells of the malignant melanoma cell line, UACC 903, were transfected with normal human genomic DNA via cationic lipofection. Transfected cultures were then exposed to R123. Surviving cells were morphologically distinct from the melanoma cells, and had the characteristics of nonmalignant cultured epithelial cells with respect to growth rate and medium requirements. Results of soft-agar cloning and the extension of this technique to other malignant cell types will be presented. Implications for the cloning of tumor suppressor genes will be discussed.

U 209 THE ROLE OF A NEW FGF-RECEPTOR IN THE DEVELOPMENT OF HUMAN LUNG TUMORS,

K. Strebhardt, A. Bräuninger, U. Holtrich and H. Rübsamen-Waigmann, Georg-Speyer-Haus, Paul-Ehrlich-Str. 42-44, 6000 Frankfurt, FRG

Using PCR we analyzed the expression of protein-tyrosine-kinases (PTK) in normal and malignant lung tissues. Specific primers for different subgroups of PTKs were subjected to PCR and gave rise to 200 bp fragments of the following PTKs: HCK, FGR, LYN, YES, PDGFB-R, CSFI-R and two previously unknown members of the PTK family: 1. CSK (intracellular PTK), 2. a new member of the family of Fibroblast Growth Factor (FGF)-Receptors: A 3,3 kb clone from a cDNA library based on RNA from lung was sequenced. The predicted aminoacid sequence contains the complete open reading frame for a new FGF-receptor which was called TKF (Tyrosine - Kinase - related to FGF-Receptors).

Screening of a variety of human tissues has shown that the TKF-gene is expressed exclusively in human lung. TKF-transcripts were not found in various lung tumors. The evaluation of TKF expression in more than 100 lung tissues indicates a role of TKF as putative diagnostic parameter for the development of this tumor. Genetic variations of TKF in human lung tumors will be discussed. Taken together these data indicate that the FGF-receptor plays an important role in the physiology of human lung.

Some tumors of gastrointestinal origin - in contrast to the corresponding normal tissues (esophagus, stomach, coecum, colon) - showed TKF expression.

U 210 ABROGATIO OF METASTATIC PROPERTIES OF NEU-TRANSFORMED CELLS BY THE INTRODUCTION OF THE ADENOVIRUS E1A GENE,
Dihua Yu, Junichi Hamada, Hong Zhang, Garth Nicolson and Mien-Chie Hung Department of Tumor Biology, University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030
Amplification/overexpression of the *neu* oncogene has been shown to correlate with the number of lymph node metastasis in breast cancer patients. However, there was no systematic study on the role of *neu* oncogene in metastasis. Here we first demonstrate that 3T3 cells transformed by mutation-activated rat neu oncogene exhibited metastatic properties by in vitro invasion assays and in vivo experimental metastasis assays, while the parental 3T3 cells did not. Since we had previously shown that the adenovirus 5 E1A gene product can suppress *neu*-induced transformation of 3T3 cells, we examined the possibility that E1A can abrogate the metastatic properties of *neu*-transformed 3T3 cells. We found that introduction of the E1A gene into *neu*-transformed 3T3 cells reduced the formation of experimental metastatic tumors. Important steps in the metastatic event are tumor cell adhesion to blood vessel and invasion of basement membrane. Therefore, we examined the ability of E1A transfectants to adhere to microvessel endothelial cells, migrate through layer of reconstituted basement membrane (Matrigel) and secrete basement membrane-degradative enzymes. Transfer of the E1A gene into *neu*-transformed 3T3 cells inhibited all three of these metastasis-associated properties. The results indicate that the E1A gene is a metastatic suppressor gene for *neu*-induced experimental metastasis and that E1A gene apparently functions by blocking multiple steps in the metastatic cascade. The molecular mechanisms by which E1A supresses *neu*-induced metastasis and transfomation is that E1A represses *neu* gene expression at the transcriptional level via a cis DNA element in the *neu* promoter.

Homologous Recombination and Positional Cloning

U 300 HOMOLOGOUS RECOMBINATION IN POLYOMA REPLICONS: EVIDENCE FOR TWO MECHANISMS,
Danielle Bourgaux-Ramoisy, Chantal Nault, Louis Delbecchi and Pierre Bourgaux, Département de Microbiologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4
RmI is a hybrid replicon consisting of polyomavirus (Py) and mouse sequences that yields unit-length polyomavirus DNA (P155) via intramolecular homologous recombination between two short direct viral repeats (S repeats). In order to assess whether P155 was the sole recombination product arising from RmI, we modified this replicon so as to render the putative reciprocal of P155 as readily detectable as P155 itself. This was achieved by introducing into RmI - specifically within its mouse component - another whole Py genome, thereby adding to the S repeats a second, much larger, internal homology. Following transfection of such constructs into permissive mouse cells, homologous recombinants were generated via one or both of two mechanisms. Conditions likely to favor transcription of one of the two viral genomes allowed the generation of a single product encompassing this genome, via a crossover occurring between S repeats (*univocal recombination*). Failure to meet such conditions however did not affect, or perhaps even enhanced, a concurrent mechanism involving the larger homologies, but yielding either one - or both - reciprocal products (*equivocal recombination*).

U 301 COSMIDS FROM HUMAN CHROMOSOME 22 DETECT MULTIPLE LOCI THAT FLANK THE DGS CRITICAL REGION,
Alisoun H. Carey *, Stephanie Halford, Robert Williamson and Peter J. Scambler, Department of Molecular Genetics and Biochem.St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, * present address: Dept. Cell and Developmental Biology, Roche Institute of Mol. Biology, Roche Research Centre, 340 Kingsland St., Nutley, N.J. 07110.
DiGeorge syndrome (DGS) is an abnormality in human embryogenesis affecting the derivatives of the 3rd and 4th pharyngeal pouches and 1st pharyngeal arch. It is well established that this disorder can be associated with monosomy 22q11, detected either by cytogenetic analysis or at the molecular level. DNA probes, shown to be hemizygous in DGS patients by quantitative hybridisation, were used to isolate cosmids from this region. The cosmids were then used for *in situ* hybridisation studies on metaphase chromosomes and interphase nuclei prepared from DGS patients with chromosomal rearrangements and normal controls. Two of the cosmids identified repetitive elements which were not detected by the clones which were used to isolate these cosmids. These repeat sequences were shown to flank the genomic region critical for DGS.

U 302 ISOLATION AND CHARACTERISATION OF NOVEL MAMMALIAN GENES CONTAINING MULTIPLE HOMEODOMAINS. Lorraine N. Clark, Jane E. Hewitt, Tracy J. Wright and Robert Williamson. Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG UK.
The homeodomain, a helix-turn-helix structure similar to the DNA-binding domains of several prokaryotic repressors and activators, was first identified in a family of *Drosophila* proteins required for proper establishment of the segmented body plan. Homeodomain proteins have since been found in a variety of vertebrates and accumulating evidence suggests that these genes play a crucial role in embryonic and fetal development. We have recently isolated two clones containing a new human homeobox gene by using a degenerate consensus oligonucleotide for helix three of the homeodomain (the most highly conserved region) to screen a chromosome 4 specific cosmid library. By hybridisation analysis, the cosmids contain at least three homeoboxes. Two of these have been sequenced, and found to be separated by only 45bp and within a single open reading frame. Southern blot analysis indicates that this gene may be part of a gene family and five distinct cross-hybridising phage clones have been isolated from a total human genomic library. One of these phage clones has been analysed in more detail and shown to also contain this "double" homeodomain. Analysis of the predicted protein structure of these linked homeodomains shows them to be divergent from the Antennapedia class (46% amino acid identity) and from the *zfh* genes of *Drosophila* (Fortini *et al* Mech. Dev. 34:113-122, 1991), which also contain more than one homeobox sequence. Homologous recombination has been used to attempt to determine the function of homeobox genes, (Chisaka and Capecchi, Nature 350:473-479). Zooblot analysis indicates that the homeobox sequences are conserved and the corresponding murine homologues are being isolated by cross-species hybridisation and by degenerate PCR. The pattern of gene expression in the mouse will be determined by Northern blot analysis, PCR amplification from RNA and *in situ* hybridisation.

U 303 GENETIC MAPPING OF A GENE CAUSING HYPERTENSION IN AN EXPERIMENTAL MODEL OF HYPERTENSION, Howard J. Jacob,[1,2] Klaus Lindpaintner,[3,4] Stephen E. Lincoln,[1] Kenro Kusumi,[1,5] Ruth K. Bunker,[5] Yi-Pei Mao,[1] Detlev Ganten,[6] Victor J. Dzau,[2] and Eric S. Lander[1,5] 1.Whitehead Institute, Cambridge, MA 02142 2.Cardiovascular Med., Stanford Univ. 3.German Inst. for High Blood Pressure Research, Heidelberg 4.Dept. of Cardiology, Harvard 5. Dept. of Biology, M.I.T. 6.Ctr for Molecular. Med. Berlin-Buch, Germany
The Stroke Prone Spontaneously Hypertensive Rat (SHRSP) is a well-characterized model for primary hypertension in humans. High blood pressure in SHRSP shows polygenic inheritance, but none of the loci responsible have previously been identified. In this general regard, hypertension is prototypical of other human diseases of complex inheritance. Genetic mapping provides a powerful strategy for the study of inherited diseases in humans: genes can be localized by linkage analysis and then cloned based on chromosomal position. However, the approach is more problematic for human diseases with polygenic or quantitative inheritance. Recently, attention has focused on the genetic dissection of complex traits in animal models as a way to identify important genes which may then be studied in human patients and families. By crossing inbred strains, one eliminates the problem of genetic heterogeneity and, by using sufficiently many animals, one can achieve the statistical power to detect genes affecting a quantitative trait. To locate genes controlling hypertension, we mapped a more than 130 DNA polymorphisms in a cross between the hypertensive SHRSP and the normotensive WKY strain. Here, we report strong genetic evidence that a gene Bp1 having a major effect (30% of genetic variance) on blood pressure maps to rat chromosome 10 with a LOD score of 5.10 and is closely linked to the rat gene encoding angiotensin-converting enzyme , which plays a major role in blood pressure homeostasis and is an important target of anti-hypertensive drugs. In addition, we are currently using the rat genetic map to look for linkage with other interesting phenotypes, which we will present at the meeting.

U 304 DERIVATION OF EMBRYONIC STEM CELL LINES FROM THE RABBIT Randall W. Moreadith, Kathy H. Graves, Molecular Cardiology Laboratories, University of Texas Southwestern Medical Center, Dallas, TX 75235
The derivation of pluripotential embryonic stem cells has ushered in a new era in molecular genetics. We report here the derivation of pluripotential ES cells from the rabbit.
Four and 5 day rabbit blastocysts were harvested from naturally mated 6-7 mo. old does. Embryos were washed and zona pellucidae mechanically removed before being placed individually in 96 well plates on fibroblast feeder layers in DMEM with 15% FBS. Six to 7 days later the embryos were trypsinized and replated onto feeder layers. Two cell types emerged as independent cell lines. The first has morphology identical to primary trophectodermal outgrowth and is capable of spontaneous vesicle formation. The second is morphologically similar to embryonic cell lines derived from the mouse and the pig. These cells appear undifferentiated in culture, are feeder dependent and are relatively slow growing. They are capable of embryoid body formation in culture and have the ability to differentiate into spontaneously contracting muscle, neuronal, epithelial and pigment producing cells.
Experiments are underway to characterize these cells further by karyotype, expression of lineage-specific genes and ability to colonize the normal embryo to produce chimeric rabbits. The availability of a rabbit embryonic stem cell will facilitate a variety of genetic experiments in an animal large enough to provide detailed physiologic measurements. Rabbit models of human genetic diseases should prove to be superior in testing novel genetic therapy.

U 305 CLONING, CHARACTERIZATION, MAPPING, AND KNOCKOUT OF A GENE ENCODING A CYTOPLASMIC PHOSPHOTYROSINE PHOSPHATASE, Bedrich Mosinger,Jr., Ulrich Tillmann, Michel L. Tremblay, and Heiner Westphal, Laboratory of Mammalian Genes and Development, National Institute of Child Health and Development, National Institutes of Health, Bldg.6, Rm.332, Bethesda, MD 20892.
Phosphotyrosine phosphatases (PTPases) are a recently identified group of enzymes that specifically remove phosphate from tyrosine residues in proteins. Two distinct types of PTPases can be distinguished, one that is located in the cytoplasm and the second that has structural features of a membrane receptor. The functions of most of these PTPases are still unknown, however their expected role in the regulation of tyrosine phosphorylation suggests that they may be involved in signal transduction, cell growth and proliferation, oncogenesis and ontogenesis. We cloned a mouse cDNA encoding a cytoplasmic PTPase (MPTPase). The MPTPase gene is expressed in many murine adult tissues with maximal expression in ovaries, testes, thymus and kidney. The homologous human cDNA (TC-PTPase) was identified previously although the human and mouse sequence differed at their 3'ends. We cloned a novel human cDNA sequence that is at its 3' end homologous to the MPTPase. We mapped the human gene to human chromosome 18p20-pter by using PCR and DNA of human/hamster hybrid cell lines.
A portion of the mouse genomic gene was cloned and used to prepare constructs for homologous recombination in mouse embryonic stem cells. Results from these experiments will be presented.

U 306 Mapping of deletions in the human immunoglobulin heavy chain constant region locus. Is non-equal homologous recombination the mechanism?

Per G. Olsson, Lennart Hammarström and C.I. Edvard Smith. Center for Biotechnology, Karolinska Institute, NOVUM, S-141 57 Huddinge Sweden.

In some cases of immunoglobulin subclass deficiencies, deletions of the corresponding gene(s) are found. Up to date, ten types of single and multiple gene deletions have been described in the human IGHC located at 14q32. The human IGHC contains two blocks of $C\gamma$-$C\gamma$-$C\varepsilon$-$C\alpha$ genes that most probably arose from a duplication. In between these blocks is a single $C\gamma$ gene ($C\psi\gamma$), of uncertain origin. The homology of $C\gamma$ coding sequences is >85%. Flanking sequences, such as 5' switch and $I\gamma$ regions are also >90% homologous. Thus, there are multiple copies of long stretches of homologous DNA in the IGHC. This probably makes the locus susceptible to non-equal homologous recombination. If recombination occurs at homologous sequences in or at the homologous genes (5 $C\gamma$, 2 $C\alpha$ and 2 $C\varepsilon$), this would result in 19 possible deletions. All deletions described up to date could be products of non-equal homologous recombinations. We have recently determined the size of a number of deletions by pulsed-field electrophoresis and the sizes comply with products of non-equal homolgous recombination. This does not, however, rule out that deletions exist that are products of other events.

The most 5' of the IGHC genes, $C\mu$ and $C\delta$, are only present in single copies and are therefore not likely to be deleted by non-equal homologous recombination. The $C\psi\gamma$ gene lacks the 5' switch region and probably other homologous 5' $C\gamma$ sequences, and the region 5' to $C\psi\varepsilon 1$ contains large deletions. These regions are thus less probable to be sites of recombinations and, in line with this, no deletions involving the regions 5' to $C\psi\gamma$ and $C\psi\varepsilon 1$ have as yet been found. No deletions including the $C\gamma 3$ gene have so far been described, indicating that regions of homology 5' of $C\gamma$ genes are not frequently involved in the deletion event. In fact, all of the hitherto described deletions can be explained by recombination of sequences 3' to the genes. This, and the fact that both $I\gamma$ and switch regions have been excluded as breakpoints in five deletion haplotypes, indicates that the switch regions are not recombination hot-spots in germ cells, although they are the site of recombination in B-cells.

U 307 LINKAGE ANALYSIS OF A FAMILY WITH FAMILIAL SINO ATRIAL DYSFUNCTION ASSOCIATED WITH QT LONG SYNDROME.

PASCAL O.*, PELETIER S.+, AUBERT D.*, HERBERT O.*, LEMARREC H.+, MOISAN J.P.*, BOUHOUR J.B.+
* Laboratory of Molecular Genetics, University Hospital HOTEL DIEU BP 1005 44035 NANTES (France)
+ Cardiologic Department, University Hospital of NANTES (France)

Familial sino atrial dysfunction is a rare disease. We described a fourth generation family of more than 50 persons (23 affected, 2 with sudden death). Electrocardiograms were realized in the cardiologic department to establish the status of each member of the family.

Blood samples were obtained from each member of the family. DNA was extracted and digested with restriction enzymes, then charged into agarose gels and transferred onto Hybond N+ filters. The filters were hybridised with 32P Labelled probes, and revealed by autoradiography.

a/ Linkage studies were performed with VNTR probes located on different chromosomes.

b/ QT Long Syndrome is associated with the disease. As described by KEATING M. and al (Science 1991 252 704-706) We performed a linkage study using Hras, and INSULIN probes.

Lod scores were calculated using LINKAGE Program and results are discussed.

U 308 INACTIVATION OF THE MAJOR MOUSE ADULT β- GLOBIN GENE (b1) BY HOMOLOGOUS RECOMBINATION CAUSES SEVERE ANEMIA IN HOMOZYGOUS ANIMALS, Ron Shehee, Paula Clark, and Oliver Smithies, Department of Pathology, University of North Carolina, Chapel Hill, NC 27514

Adult mouse β-globin is encoded by two genes, b1 and b2. b1 is responsible for most (60-80% depending on the strain and individual) of the adult β-globin. We have inactivated the b1 gene by homologous recombination in embryonic stem (ES) cells. Mice homozygous for this inactivated gene are anemic and die perinatally. These homozygous mice seem to have much more severe anemia than mice homozygous for a naturally occurring deletion of the b1 gene (Skow *et al.*, 1983, Cell **34**, 1043). The numbers of homozygous mice born from heterozygous matings is also much lower than the 25% expected. We will discuss the mutant phenotype and penetrance observed in b1/b1 minus mice.

U 309 ISOLATION OF TGF-β1 INDUCED GERM-LINE RNA FOR IgA. *C. I. Edvard Smith, Khalid B. Islam, Lars Nilsson, Lennart Hammarström & Paschalis Sideras.* Dept. of Clinical Immunology and Center for BioTechnology, Karolinska Institute, S-141 86 Huddinge, Sweden and Unit for Applied Cell and Molecular Biology, Umeå University, Umeå, Sweden.

The mechanism underlying IgA deficiency is unknown, but in addition to an influence of HLA (*Nature* 347:289, 1990), we have recently demonstrated that the immunoglobulin heavy-chain locus in 14q32 is associated with IgA deficiency (submitted). In mice TGF-β1 has previously been demonstrated to induce the synthesis of IgA, presumably through an effect on isotype switching. In mice TGF-β1 induces production of sterile transcripts devoid of a variable portion, but instead, containing a novel exon located upstream the switch a region. As this transcription together with conformational changes of chromatin in this chromosomal region may constitute the very first step in IgA synthesis, we set out to isolate the corresponding human sequences. Cultivation of human spleen cells in the presence of *B Catarrhalis* and human TGF-β1, enabled us to identify truncated transcripts originating from upstream both the switch α1 and α2 regions, as demonstrated by the analysis of cDNA and cosmid clones. Germ-line mRNA for both the secreted and the membrane form of IgA was identified. These transcripts contained novel exonic regions designated Iα regions according to the generally accepted nomenclature. Comparison with the corresponding mouse Iα region demonstrated a very low homology of the coding sequences. In contrast, the promoter regions contained highly conserved elements, indicating that these regions are functionally important. We are presently studying the expression of these regions in individuals with immunoglobulin deficiencies.

Positional Cloning

U 400 HOMOZYGOSITY MAPPING IN THE LOCALIZATION OF THE FMF GENE, Ivona Aksentijevich, Elon Pras, Luis Gruberg, Leandrea Prosen, Mordechai Pras, and Daniel Kastner. Genetics Unit, Arthritis and Rheumatism Branch, NIAMS, NIH, Bethesda, MD, and Department of Medicine F, Sheba Medical Center, Tel Hashomer, Israel.

Familial Mediterranean Fever (FMF, MEF) is an autosomal recessive disorder characterized by recurrent attacks of fever, synovitis, peritonitis, or pleurisy. Using conventional linkage analysis we have studied DNAs from 35 Israeli families to map the FMF gene to the short arm of chromosome 16. Generally, the rate of consanguinity within FMF families is much higher than that of the population at large. In our panel, 14 families were inbred, including 8 first cousin marriages (19 affected offspring) and two uncle-niece marriages (3 affected offspring). We took advantage of this high rate of consanguinity in our pedigrees to examine homozygosity at marker loci, as proposed by Lander and Botstein (Science 236:1567). For the the two allele marker D16S84 (maximum lod = 10.45, θ = .05), 6 of 6 informative affected offspring of first-cousin marriages, and 3 of 3 informative offspring of uncle-niece marriages, were homozygous. For the VNTR probe 5'HVR (maximum lod = 14.31, θ = 0.07), 11 of 13 informative offspring of first-cousin marriages, and 2 of 3 informative offspring of uncle-niece marriages, were homozygous. In contrast, for the 108 markers that we examined and rejected before finding linkage to chromosome 16, only two showed more homozygous affected persons than expected on the basis of chance. Moreover, we had identified an area of chromosome 17q that included five loci with moderately positive two point lod scores (1.5 - 2.5), but for which only one of 16 affected inbred persons were homozygous. The fact that we ultimately rejected this area of chromosome 17 confirms the value of "homozygosity mapping" as a strategy for locating genes that cause recessive diseases.

U 401 CONSTRUCTION OF A YAC CONTIG FOR A REGION OF THE MOUSE X CHROMOSOME HOMOLOGOUS TO HUMAN Xq27-Xq28. A. Chatterjee, C. J. Faust, and G. E. Herman, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX, USA

Our laboratory has developed detailed genetic and/or physical maps in the region of the mouse X chromosome homologous to human Xq27-Xq28, an area rich in disease genes. These analyses suggest a gene order of *(Cf-9)-(Fmr-1)-Ids-(Gabra3, DXBay10)-(DXMIT1, F8a, DXPas8, Bpa)-(DXBay2, L1Cam)-Rsvp-Gdx-P3-G6pd-(Cf-8)-Dmd*. *DXBay10* defines an anonymous human locus conserved in several species; the probe *F8a* is a mouse clone isolated with the human factor 8 intron 22 cDNA. Bare patches *(Bpa)* is an X-linked dominant mutation that is lethal in males. *DXMIT1* is a murine microsatellite that includes a dinucleotide repeat. We have begun to isolate murine YACs in this region using PCR to detect specific target DNA sequences and have constructed a contig extending 600-700 kb including the loci *F8a*, *DXPas8*, and *DXBay2*. Nine additional YACs at the loci *Gabra3* and *DXBay10* have been isolated. We are continuing to walk toward *Rsvp* and will attempt to incorporate *Gabra3* and *DXBay10* into the contig. Once a complete contig has been constructed, we will be able to isolate genes using a variety of strategies and identify homologous human X genes which may represent disease loci. A complete map for this region of the mouse X chromosome would also help to fill gaps in YAC contigs for the corresponding region of human Xq28.

The authors thank W. Dietrich, E. Lander, S. Kenwrick, B. Levinson, and J. Gitschier for providing several of the DNA probes used in this study.

U 402 RADIATION HYBRIDS TO CLONE AND MAP DNA MARKERS SPANNING THE INCONTINENTIA PIGMENTI 1 (IP1) LOCUS. Jerome L. Gorski, Eric L. Reyner, and Eric N. Burright, Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109.

The isolation and localization of DNA markers from a specific targeted chromosomal region has been a necessary prerequisite for the positional cloning of human genes and the construction of regional maps. As part of an effort to clone the incontinentia pigmenti 1 (IP1) locus, we sought a strategy to increase the efficiency of isolating and mapping region-specific DNA markers from Xp11.21. To accomplish this task we performed two complementary experiments using X-chromosomal radiation hybrids (RHs). First, RHs were identified which contained DNA markers spanning IP1 X-chromosomal translocation breakpoints (BPs). RH DNA containing these loci was used to perform human-specific IRS-PCR amplifications and DNA products were mapped to Xp11.21 using a RH mapping panel. Second, 67 X-chromosomal RHs were constructed and used, in conjunction with a IP1 somatic cell hybrid mapping panel, to construct a RH map of region Xp11.21-p11.1. Using this strategy, 3 different DNA markers were isolated which mapped to a region between IP1 tBPs and an order for 12 DNA loci spanning the IP1 tBPs was predicted: pter-DXS146-ALAS2-(DXS323, probe 2:30)-(IP1 tX;13, IP1 tX;9)-DXS705-DXS706-MTHFDL1-DXS420-DXS14-DXS343-(DXS370, probe p49a)-IP1 tX;17-DXZ1(cen). To further verify the order of these markers, we have used pulsed-field gel electrophoresis (PFGE) to construct a physical map of this region. This map links DXS370 to DXS705 on contiguous restriction fragments spanning 3.5 Mbp; this region contains at least 5 HTF islands. Our results indicate a marked gradient in the retention of pericentromeric DNA markers among RHs; 71% of the RHs retained DXZ1 while 30% retained DXS146 with intervening DNA markers retained at intermediate levels.

U 403 PROGRESS TOWARDS IDENTIFYING THE GENES RESPONSIBLE FOR X-LINKED SEVERE COMBINED IMMUNODEFICIENCY (X-SCID) AND X-LINKED AGAMMAGLOBULINEMIA (XLA)

Christine Kinnon, Marie-Anne O'Reilly, Alison Jones, Lesley Alterman, Sue Malcolm and Roland Levinsky, Division of Cell and Molecular Biology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, U.K.

The molecular bases of the X-linked immunodeficiency diseases remain largely undetermined. We are interested in identifying and isolating the genes responsible for causing two of these diseases, namely X-SCID and XLA. X-SCID is characterized by absent cell-mediated and humoral immunity and is usually fatal by 1 to 2 years of age if not treated by bone marrow transplantation. XLA is characterized by agammaglobulinemia involving all immunoglobulin subclasses and is the direct result of a lack of mature B cells. Since almost nothing is known about the protein products of these genes we have, therefore, relied on a "positional cloning" approach to identify candidates for these genes.

We have made substantial progress in that we have constructed genetic and physical maps of the regions known to contain these loci. Currently, our long-range physical maps of the X-SCID locus in Xq13-21.3 cover nearly 5Mb of genomic DNA and encompass the four probes (DXS325, DXS347, DXS441 and PGK1) that show no recombinations with the disease locus in more than twenty informative meioses. In the case of XLA, we have constructed physical maps that extend over 5Mb in Xq22 and encompass the distal genetic flanking markers DXS94 and DXS17. In addition, we have identified at least four putative CpG islands in the region of DXS178, the locus which shows no recombination with the disease in over thirty informative meioses. We are now moving on to refine these maps, to clone these regions of DNA using overlapping cosmid and YAC clones, and to isolate candidate genes in these regions. We will present genetic and physical maps of these loci and summarize the progress we have made so far.

U 404 AUTOMATING GENOMIC DNA PURIFICATION FROM SMALL SAMPLES. Sandy M. Koepf, Applied Biosystems, 850 Lincoln Ctr. Dr., Foster City, CA. 94404

With the advent of PCR technology, the sample size needed for DNA analysis can be much smaller than for traditional DNA assays. This allows samples such as small amounts of blood (20-50 µL) and submicrogram quantites of tissue to be readily analysed. Sample handling, however becomes more critical because the amount of sample is limited. We have focused on automating genomic DNA purification from small samples on the GENEPURE™ Nucleic Acid Purification System from Applied Biosystems. The purification can be done with either organic extraction or non organic purification (Fast Cycle Chemistry) depending on the sample and the needs of the researcher. But in either case, BaseBinder™ resin is added during the precipitation stage to increase the recovery of small amounts of genomic DNA (<1µg). There are several advantages to the GENEPURE™ over manual methods or to non purification strategies, reliability and reproducibility are just a few.

U 405 CHARACTERIZATION OF THE MOUSE CORTICOSTEROID BINDING GLOBULIN cDNA AND THE GENE Mauri M. Orava, Carolyn L. Smith and Geoffrey L. Hammond, MRC Group in Fetal and Neonatal Health and Development, University of Western Ontario, London, Ontario, N6A 4L6, Canada

Corticosteroid binding globulin (CBG) is the major plasma transport protein for glucocorticoids in vertebrate species. In addition, CBG may have other physiological functions. It belongs to the serine proteinase inhibitor (SERPIN) superfamily and acts as a substrate for neutrophil elastase. Cleavage of CBG by neutrophil elastase releases steroid from the binding site, and this may be important for the delivery of glucocorticoids to sites of inflammation. Large changes in CBG gene expression during the fetal period suggest a specific role for CBG during development. In both humans and rodents, genetic variants of CBG with abnormal steroid binding have been characterized, but the association between these variants and disease states is unclear.

To study the evolution, genetics and function of CBG, we have cloned and characterized a mouse CBG cDNA and its gene. A full-length 1476 bp cDNA consisted of 5' non-coding region of 54 nucleotides, followed by a 397 amino acid open reading frame and a 228 bp 3' non-coding region. Homology to the human and rat cDNA is 58 % and 79 % at the amino acid level, and 73 % and 88 % at the nucleic acid level, respectively. The mCBG gene is spread over 10 kb and contains five exons. The first exon codes for 69 bp of 5' untranslated sequence, whereas exons II-V are 145 - 611 bp in size and contains the entire coding sequence. Analysis of the 5' flanking region of the gene revealed sequences that resemble TATA- and CAAT-box motifs, as well as consensus sequences for the binding of several liver specific transcription factors. Southern analysis of the genomic DNA suggested that mouse CBG is the product of a single gene. The gene was mapped to chromosome 12 in the vicinity of the alpha1-proteinase inhibitor (A1-PI) gene(s), another member of the SERPIN superfamily. The close chromosomal location and high sequence homology between mouse CBG and A1-PI suggest that these genes have a common evolutionary origin.

U 406 NEW SHORT TANDEM REPEAT POLYMORPHISMS ON HUMAN CHROMOSOME 22, Joanne C. Porter and Jennifer M. Puck, Human Genome Center for Chromosome 22, Dept. of Pediatrics, Children's Hospital of Philadelphia, and University of Pennsylvania, Philadelphia, PA 19104

Short tandem repeat (STR) polymorphisms occur frequently and often have high information content with many alleles, making them ideal for genetic mapping. A systematic search for new STR's on chromosome 22 (CH22) has been initiated, using a flow sorted CH22 phage library, LL22NL02 (ATCC 57779), subcloned into pBluescript as BstY1 fragments with an average size of 1 kb. Twenty poly-TG clones with inserts <2 kb have been selected for sequence analysis to develop primers flanking the target STR. PCR analysis of 4 clones to date has confirmed their position on CH22 using a mapping panel of human/rodent hybrids containing CH22. Human genomic DNA from 5 CEPH pedigrees, amplified with end-labeled primers, has demonstrated polymorphism in each of these STR's. An amplification product of 283 bp from clone 9.11, with a complex series of short runs of TG units, has only 2 alleles in 22 CH22s tested. The heterozygosity is about 16%. In contrast, clone 35.12, which has a PCR product of 192 bp, is highly polymorphic with 6 different alleles and a heterozygosity of about 80%. Primer sequences are:

9.11: AC strand: GAGAAGAGTGTAAGTGCACC
TG strand: GACCCTGAGCTGTTTCACTGG

35.12: AC strand: TGACAACAACCATCAAGTCCA
TG strand: GGAGCTGCATGTACTAGCTGG

PCR was carried out in 25 µl using 0.1-1 µg genomic DNA and 13 pmoles of primers, with a 5 min initial denaturation followed by 30 cycles of 62°, 75°, and 95°, in 0.25 mM dNTP; 10 mM Tris, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$.

U 407 LINKAGE OF THE GENE FOR FAMILIAL MEDITERRANEAN FEVER TO THE SHORT ARM OF CHROMOSOME 16, Elon Pras, Ivona Aksentijevich, Luis Gruberg, Leandrea Prosen, Michael Dean, Mordechai Pras, and Daniel Kastner. Genetics Unit, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD; PRI/Dyncorp, Frederick Cancer Research Facility, Frederick, MD; and Department of Medicine F, Sheba Medical Center, Tel-Hashomer, Israel.

Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever, abdominal pain, arthritis, and pleurisy; some patients eventually develop systemic amyloidosis. The biochemical basis for this disorder is unknown. Using DNAs from 35 Israeli families, mostly of North African and Iraqi Jewish origin, we conducted a genome-wide search for the FMF locus. Here we present data mapping this disease to the short arm of chromosome 16. For the four markers D16S82 (p41-1/SacI), D16S84 (pCMM65/TaqI), D16S83 (pEKMDA2-1/RsaI), and HBA (5'HVR/RsaI) we obtained maximum lod scores of 2.71 (θ = 0.07), 9.66 (θ = 0.04), 9.35 (θ = 0.03) and 14.31 (θ = 0.07), respectively. Multipoint analysis in the non-Ashkenazi Jewish families with D16S84 and HBA as fixed loci gave a maximum lod score of 19.86 centromeric to D16S84. Crossovers defined by the 16p markers mentioned above place the FMF gene in an interval of about 7 cM between D16S83 and D16S82. Currently we are trying to localize the gene more precisely using additional markers spanning this area. This is the first step in the isolation and characterization of the FMF gene.

U 408 THE CYTOCHROME b_5 GENE IS LOCATED ON CHROMOSOME 18. Alan W. Steggles and Sara J. Giordano, Dept. of Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

In most species cytochrome b_5 (b_5) exists either as a soluble 97aa protein found in the erythrocytes, or as a membrane bound 133aa protein in liver and other tissues. One of the roles of b_5 is to act with cytochrome b_5 reductase to convert methemoglobin back to hemoglobin, thereby maintaining oxygen transport in blood. Deficiencies in either of these two proteins leads to the genetic disease, inherited methemoglobinemia. We have recently shown that in humans the liver b_5 mRNA is derived from the erythrocyte b_5 mRNA by alternative splicing, and have partially characterized the b_5 gene. During the screening of genomic libraries we isolated and characterized three psuedogenes for b_5, each one contained within a pair of repeat sequences. We also isolated a cDNA derived from a fourth transcribed psuedogene during the screening of a reticulocyte cDNA library in λZAP. Using a Southern blot derived from a series of human-hamster cell hybrids (Bios Ltd.) we localized the b_5 gene and the four psuedogenes to individual chromosomes. Cytochrome b_5 is located on chromosome 18, and one of the psuedogenes is located on chromosome 20. The other three, including the transcribed psuedogene, are located on chromosome 14, and appear to be within 40 kb of each other. The presence of the four (or more) b_5 psuedogenes will make the analysis of b_5 defective patients more difficult. Supported by funds from the United Way, Stark County, and the American Heart Association, National and Ohio Affiliates.

U 409 DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) ANALYSIS OF CANDIDATE GENES FOR SCHIZOPHRENIA, James T. Warren Jr., Michael L. Peacock, and John K. Fink , University of Michigan Department of Nerurology, 1103 E. Huron, Ann Arbor,MI 48104

We have used DGGE to identify mutations in the coding regions of genes which have been pharmacologically implicated in schizophrenia. Through the use of theoretical melt maps and "GC clamped" PCR primers, overlapping fragments of the D1 and 5HT1A receptor gene sequences have been designed for which point mutations would yield DGGE detectable polymorphisms. The initial analysis of 18 schizophrenic probands and 20 normals has revealed 4 distinct polymorphisms in different regions of these 2 candidate genes. DGGE analysis coupled with direct sequencing of polymorphic regions have proven to be rapid and reliable means for detecting mutations in candidate genes and determining their association with schizophrenia.

Gene Transfer, Gene Therapy, and Gene Expression

U 500 *Abstract Withdrawn*

U 501 ABNORMAL EXPANSION AND EXTENDED SURVIVAL OF T-CELLS IN THE HUMAN *bcl*-2/Ig ENHANCER TRANSGENIC MICE. *Makoto Katsumata*, Richard M. Siegel*, Diane C. Louie*, Toshiyuki Miyashita*, Yoshihide Tsujimoto†, John C. Reed*, Peter C. Nowell* and Mark I. Greene*. *Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; †The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.* We have generated transgenic mice bearing the human B cell lymphoma/leukemia-2 (*bcl*-2) gene with the human immunoglobulin (Ig) heavy chain enhancer. This construct mimics the chromosomal translocation t(14;18)(q32;q21) which juxtaposes the *bcl*-2 proto-oncogene with the immunoglobulin heavy chain locus and is found in about three fourths of human B cell follicular lymphomas. Our transgenic mice have shown similar results to other groups with extended survival and accumulation of peripheral B cells. However, one of the transgenic mouse lines consistently demonstrated abnormal T-cell expansion and a chronic lymphocytic leukemia (CLL)-like phenotype possibly due to an integration site effect. Western blotting analysis has shown that a large amount of the *bcl*-2 protein is produced in the thymus of these mice. After 15 weeks of age, the proportion of thymocytes with a mature phenotype was 4-39 times higher than that of negative controls. When cells isolated from spleen and lymph nodes were placed in culture, the T-cells did not proliferate spontaneously. However, the number of T-cells that survived after twelve days of culture was 25-54 times higher than that of age matched controls. This line of transgenic mice may be useful as an experimental model for steroid treatment of leukemia and for the study of the mechanisms of thymic selection and apoptotic cell death during T-cell development.

U 502 MECHANISM OF HEMOGLOBIN INDUCTION IN ERYTHROLEUKEMIA CELLS: A NEW IN VIVO FOOTPRINTING METHOD, In-Hoo Kim*@,Kyung Chin*, Patricia Berg**, Alan N. Schechter* and Griffin P.Rodgers*, *Laboratory of Chemical Biology, NIDDK, NIH, Bethesda, MD20892; **University of Maryland, School of Medicine, Baltimore, MD 21201; @College of Medicine, Dong-A University, Pusan, Korea;
Several lines of clinical and experimental evidence suggest that elevated levels of fetal hemoglobin may improve the clinical course of individuals with sickle cell disease and β-thalassemia. A number of cytotoxic drugs have been shown to enhance γ-globin synthesis *in experimental animals* and patients with hemoglobinopathies through mechanisms involving transcriptional regulation. The K562 human erythroleukemia cell line shows constitutively low levels of embryonic and fetal but not adult hemoglobin,and can be reversibly induced to increase γ-globin gene expression in response to hydoxyurea and other agents. We are therefore using the K562 cell as a model system to understand the mechanism of induction of globin gene transcription. K 562 cells have been grown in the presence of 25μM hemin and 100μM hydroxyurea. The status of *in vivo* protein-DNA interactions in the promoter region of the Gγ gene was investigated by a new ligation-mediated polymerase chain reaction (LMPCR), using N-ethyl,N-nitrosourea(ENU). We find that ENU acts efficiently on suspension cells and can detect protein-DNA interactions not revealed by the commonly used dimethyl sulfate(DMS) method. In the cells induced by hemin or hydroxyurea, several consensus sequences 5' of the Gγ gene were extensively protected, including CACCC, CCAAT, GATA-1, ATAAA and octamer sequences. In contrast, these functionally important sequences have not consistently been found to be protected in studies utilizing conventional *in vitro*DNA footprinting or DMS treated *in vivo* footprinting. Moreover, we have observed additional protected sequences further upstream (>250bp) with the ENU method. We conclude that our newly developed ENU method may be more sensitive than that using the DMS approach, especially in the detection and characterization of weak DNA-protein binding interactions which may be involved in the induction of globin gene transcription by pharmacological agents.

U 503 v-rel INHIBITS MURINE c-myc EXPRESSION THROUGH NFkB ELEMENTS
Francis A. La Rosa, Douglas B. Spicer, Mabel P. Duyao, Dana J. Kessler and Gail E. Sonenshein, Dept. of Biochemistry, Boston University School of Medicine, Boston, MA 02113
c-myc is an early response gene associated with cellular proliferation. We have shown the murine c-myc gene to contain two functional NFkB binding sites. They are located -1101/-1081 b.p. (URE) and +440/+459 b.p. (IRE) with respect to the P1 promoter. *Upon isolation of the clones of the* NFkB subunits it was discovered that the N terminal portions share distinct homology with the oncogene v-rel. v-rel transfection down-regulated activity of NFkB elements. Also, expression of v-rel has been shown to be anti-proliferative in NIH 3T3 fibroblasts. This anti-proliferative effect along with the observed inhibition of transactivation by NFkB led us to test the ability of v-rel to down regulate c-myc gene transcription. Cotransfection of v-rel with 2 copy URE- or 4 copy IRE- TK-CAT constructs severely decreased CAT activity by up to 25 fold below levels observed with cotransfection of the parental vector. Furthermore,the v-rel expression vector down regulated activity of murine c-myc promoter- CAT constructs by 5 to 10 fold. Upon mutation of the two NFkB binding sites within the murine c-myc-CAT construct, such that NFkB is no longer able to bind, v-rel down regulation was lost. Gel shift experiments using nuclear extracts from v-rel transfected cells showed that a complex was induced, suggesting the increase of a negative factor. Upon specific mutation of the NFkB binding sites the complexes were no longer able to bind. This data suggests the idea that the cytopathic effects of v-rel on NIH 3T3 fibroblasts is mediated in part by the down-regulation of c-myc expression through its two NFkB elements.

U 504 TOWARDS SOMATIC GENE THERAPY FOR mut METHYLMALONIC ACIDEMIA (MMA) AND PROPIONIC ACIDEMIA (PA). Fred D. Ledley, Jozsef Stankovics, Takako Sawada, Mike Wilkemeyer, R. Mark Adams, Humberto E. Soriano. Howard Hughes Medical Institute, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030
The considerable morbidity and mortality of mut° MMA and PA make these disorders potential candidates for somatic gene therapy. The cDNAs for methylmalonyl CoA mutase (MCM) and alpha-propionyl CoA carboxylase (PCCA) have been cloned, and DNA-mediated gene transfer of these clones into deficient cells has shown to reconstitute propionate metabolism to normal levels. The design of gene therapy for these disorders requires attention to both methods of gene delivery and the biological effect of genetic reconstitution. The liver is the preferred target for gene therapy of MMA and PA as the major site of normal propionate (propionyl CoA) metabolism. Also, the capacity for propionate flux is >10 times higher in hepatocytes than other primary cells, and overexpression of MCM or PCCA does not increase propionate flux. Amphotropic retrovirus carrying human MCM has been constructed which will correct the defect in mut fibroblasts, efficiently transduce primary human hepatocytes, and direct transcription of the MCM gene in these cells. We demonstrate that primary human hepatocytes can be transplanted into SCID mice and engraft within the hepatic parenchyma. We have performed hepatocyte transplantation in a baboon model where up to 5% of host hepatocytes can be constituted from an autologous graft. The question remains whether a fraction of cells with MCM or PCCA activity would have a therapeutic effect. Clinical data suggests that mutations with 1-5% normal MCM activity prevent the worst consequences of MMA. In vitro studies show that restoration of [^{14}C]-propionate metabolism in transduced cells is proportionately greater than the fraction of cells transformed, suggesting that metabolites are cleared from transformed and non-transformed cells. Also, the products of [^{14}C]-propionate metabolism can be identified in both transformed and non-transformed cells. In the absence of a homologous animal model for MMA or PA, these studies support the feasibility of performing somatic gene therapy by partial hepatectomy, ex situ transduction of hepatocytes, and autologous transplantation.

U 505 HYPOMYELINATION IN TRANSGENIC MICE CARRYING A MYELIN BASIC PROTEIN C-MYC GENE CONSTRUCT. Jacqueline M Orian[1], Andrew W.S. Mitchell[1], Wendy E. Marshman[1], Michael F. Gonzales[2] and Andrew H. Kaye[1]. Department of Surgery (RMH), Melbourne University, Parkville, Victoria, Australia 3052[1]. Department of Anatomical Pathology, Royal Melbourne Hospital, Parkville, Victoria, Australia 3050[2].
Transgenic mice carrying a construct consisting of a 1.3 kb mouse myelin basic protein (MBP) promoter fused to a 3.4 kb fragment of human c-myc gene (containing exons 2 and 3 only) were produced. In one pedigree denoted J02-50, mice exhibited a shivering phenotype from about two weeks of age, suggesting abnormalities in myelination. Since the peak of myelination in mice occurs between 18 and 21 days, mice of that age group were analysed using non-transgenic littermates as controls. No obvious abnormalities were observed at the histopathological level, however analysis by immuno-gold histology, Northern blotting and electron microscopy revealed that very low levels of myelin basic protein were present and that myelin sheaths were absent in these mice. Mice exhibiting this phenotype survive and are able to breed. Therefore, a time course experiment was performed using mice aged between 10 and 56 days to investigate the process of myelination in J02-50 mice. Mouse brains were analysed (a) by histochemical techniques, (b) by immuno-gold histology (c) by Northern blotting and (d) by electron microscopy. Data obtained to date show that myelination does occur in J02-50 mice but at a very slow rate. Myelination is incomplete even at 56 days of age. Examination of peripheral nerves has shown no differences between transgenic and non transgenic mice. It would appear therefore that J02-50 mice suffer from a phenomenon of delayed myelination which is confined to the central nervous system. J02-50 mice may represent a useful model for the study of myelin formation.

U 506 GENE TRANSFER TO MAMMALIAN CELLS USING ACCELL™ TECHNOLOGY, W.F. Swain, D.E. McCabe, J.R. Haynes, and N.-S. Yang, Agracetus, Inc., 8520 University Green, Middleton, WI 53562

We have developed a method for gene transfer to a wide variety of cells (McCabe, et al. (1988) BioTechnol. 6: 923-926; Yang et al. (1990) PNAS 87: 9568-9572) by coating DNA onto gold microparticles and accelerating these particles into target cells. Expression of introduced genes has been demonstrated in mammalian tissue culture cells, murine and human primary cells, organ explants, and a variety of tissues *in situ*. Stable transformation is obtained at frequencies around 10^{-3} to 10^{-4} in CHO and MCF-7 cell cultures, respectively. Being physical, the method is expected to be generally applicable, and has proven effective for all cell and tissue types tested thus far. This technology may therefore be useful for applications of gene therapy.

U 507 TRANSGENIC MICE HARBORING HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE EXHIBITED NEONATAL ABDOMINAL HEMORRHAGE, Myrna E.Trumbauer, Susan H. Socher, Kathryn J. Hofmann, Michael W. Conner and Howard Y. Chen, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, N.J. 07065

Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of tissue-type plasminogen activator (t-PA) and urokinase (u-PA) in normal human plasma. To investigate the physiologic and pathophysiologic roles played by PAI-1 we developed mice that are transgenic for PAI-1 through the pronuclear microinjection of a linear 2.2 kb kpn1 fragment containing mouse metallothionein-1 (MT-1) promoter and human PAI-1 cDNA with bovine GH poly A sequences into fertilized one-cell mouse embryos. Morphologic abnormalities observed during the early postnatal period include scaly skin, swollen limbs and necrotic tails, similar to that previously reported (Erickson et al, Nature 346:74,1990). Histologic examination showed hyperkeratosis of the skin, edema and hemorrhage in the limb and epithelial necrosis in the tail. No actual fibrin clots were detected, however, which suggests that their presence might be ephemeral. Analysis of plasma samples showed that increasing levels of hPAI-1 correlated with increasing severity of symptoms in transgenic mice. Administration of zinc in the drinking water for the pregnant mice resulted in live births. However, some transgenic pups developed severe symptoms of abdominal hemorrhage and died within two days. Similarly treated control pregnant females gave birth to healthy pups without symptoms. Some progenies produced from matings between hemizygous transgenic parents also exhibited abdominal hemorrhage without zinc treatment. Although these symptoms look surprisingly similar to neonatal bleeding reported for u-PA trangenic mice which exhibited prolonged clotting times (Heckel et al, Cell 62:447, 1990), blood sample analyses showed no deficiency of the clotting factors in the PAI-1 transgenic mice. Further studies are required to delineate the mechanism for abdominal hemorrhage in these mice.

U 508 DEVELOPMENTAL EXPRESSION OF Bcl-2, A PROTEIN INVOLVED IN PROGRAMMED CELL DEATH. Deborah Veis, Stanley J. Korsmeyer, Department Medicine, Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

Bcl-2 was identified as an overexpressed gene following translocation into the Ig locus in follicular B cell lymphomas. Bcl-2 is normally expressed at high levels in pre B and activated B cells, but at low levels in most quiescent and terminally differentiated B cells. Bcl-2 protein is also expressed in a variety of mature tissues outside the B cell lineage including T cells in the thymic medulla, stem cells in skin and intestinal epithelia, glandular breast cells, and some post-mitotic neurons. Transgenic and cell line studies indicate a novel role for bcl-2 as an antidote to certain programmed cell death pathways. Since programmed cell death may play a significant role in development, we decided to examine the expression of bcl-2 during murine embryogenesis.

S1 nuclease protection assays of RNA derived from a mouse embryonic stem cell line D3, and from 12 and 15 day post-conception embryos show that bcl-2 expression begins at an early age. Examination of RNA from individual organs at day 18 reveals that expression is widespread.

A hamster anti-mouse bcl-2 antibody was raised by immunizing Armenian hamsters with murine bcl-2 protein produced in a bacterial expression system. This antibody, 3F11, is monospecific for bcl-2 protein on Western blots and in situ on frozen tissue sections. In embryonic tissues (e.g. at days 14.5 to 17.5) bcl-2 is expressed widely, yet there is a distinct spectrum of expression in defined cell populations. For example, there is higher expression in the cortical neuroblast layer of brain than in deeper layers. In the lung, cells lining large airways express much higher levels than neighboring cells. Sensory epithelia (otic, nasal, and retinal) also contain high levels in contrast to surrounding supporting tissues. However, the expression of bcl-2 in the retina is much more restricted in the adult than in the 14.5 and 17.5 day embryos.

Bcl-2 expression in adult tissues is limited to zones of long-lived or progenitor cells in tissues which are susceptible to apoptosis. The widespread expression of bcl-2 in the embryo may indicate that the prevention of programmed cell death is a substantial developmental paradigm.

DNA Repair, Imprinting, Eye Disorders

U 600 ONE OR TWO GENES FOR XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP A?
James E. Cleaver, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750.

Xeroderma pigmentosum (XP) is a human hereditary disorder affecting repair of ultraviolet (UV) damage to DNA. Cellular complementation has led to the designation of 7 XP groups (A-G) that are defective in nucleotide excision repair. The two major UV photoproduct classes, cyclobutane dimers and (6-4) photoproducts, are not processed in identical fashion. We have found that (6-4) photoproducts, in contrast to dimers, are formed preferentially in regions containing active genes, which explains in part their more rapid repair. An XP group A revertant we developed has normal UV resistance; this cell line excises (6-4) photoproducts but does not excise dimers from the whole genome or from an actively transcribed gene (dihydrofolate reductase, dhfr) after high UV doses (26 J/m^2). Human cells can therefore under some conditions discriminate between the two major photoproduct classes, and (6-4) photoproducts may play a greater role than dimers in lethal effects of UV light. Quantitative PCR amplification of cDNA from the XPAC gene on chromosome 9 showed reduced message level in XP group A cells, but normal levels in the XPA revertant. The original XPAC gene had a chain terminating mutation (C→T) in codon 207; the revertant had a further change in codon 207 (T→G) which restored the reading and resulted in a substitution of glycine for arginine in the XPAC gene product. This observation has two alternative interpretations, both attribute to the XPA gene product a role in primary photoproduct recognition. Either (a) an amino acid change in this gene permits recognition of (6-4) photoproducts but not dimers, or (b) the change may be a null mutation and this gene normally codes for a (6-4) recognition protein, dimers being recognized by a different gene product. The latter interpretation would require two separate genes for XP group A. This can be reconciled with the classification of XP into complementation groups since these are defined by the restoration of unscheduled DNA synthesis in cell hybrids using short labeling times which pertains more to the genetic regulation of (6-4) repair than to dimers. Work supported by OHER, U.S. Dept. of Energy, contract no. DE-AC03-76-SF01012.

U 601 MOLECULAR PROBE ANALYSES OF 20 PRADER-WILLI SYNDROME (PWS) FAMILIES
Li-Wen Lai, Suzanne B. Cassidy and Robert P. Erickson, Steele Memorial Children's Research Center, Section of Genetics/Dysmorphology, Department of Pediatrics, University of Arizona Health Science Center, Tucson, Arizona, AZ 85724

Molecular diagnosis of PWS is important because clinical diagnosis is frequently difficult and cytogenetic analysis may not detect the characteristic 15q11-13 deletion in up to 40% of PWS patients. We performed molecular diagnoses in 20 patients suspected of having PWS. 18 patients were cytogenetically normal, and 2 patients were found to have 15q11-13 deletions by cytogenetic analysis, but had atypical presentations of PWS. Probes in Prader-Willi critical region (PWCR), D15S9, D15S10, D15S11, D15S12, D15S13, D15S24 and a terminal 15q VNTR probe pMS 620 were used in RFLP analysis. In addition, D15S9 was used to detect differential methylation patterns in the PWCR region since DNA methylation patterns within this locus were found to be different between maternal and paternal chromosomes (Driscoll et al. 1991, AJHG suppl. 49:334). Maternal disomy was identified in 9 patients including a clinically typical PWS patient who had been shown prenatally to have trisomy 15 by chorionic villus sampling and postnatally to have maternal disomy of chromosome 15 (Cassidy, Lai and Erickson et. al., submitted to AJHG). Submicroscopic deletions in the PWCR were found in 3 patients. The remainder, including the 2 patients with cytogenetically detectable 15q11-13 deletion, did not have either maternal disomy or submicroscopic deletion of PWCR or were not informative. These RFLP results were confirmed by the differential methylation patterns using the D15S9 probe. Our results with the methylation method were consistent with those reported by Driscoll et al, and supported their suggestion that studies of methylation are a useful alternative to RFLP for molecular diagnosis of PWS, particularly when parents' samples are not available. In conclusion, molecular methods provide a great advantage in diagnosing patients with PWS.

U 602 THE GENETIC AETIOLOGY OF TRISOMIES 13 AND 18, Siobhan C. Loughna and Gudrun E. Moore, Institute of Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, Royal Postgraduate Medical School, London, W6 OXG, U.K.

Human trisomies 13 and 18 occur in approximately 1 in 7000 and 1 in 5000 live births respectively. They are both compatible with life but prolonged survival is rare. Abnormalities include cleft lip and/or palate, congenital heart disease, holoprosencephaly and growth retardation for trisomy 13. Defects in trisomy 18 include congenital heart disease, rocker bottom feet, polyhydramnios and growth retardation.

The presence of an extra chromosome 13 or 18 results in these abnormal phenotypes. Consequently the probable over-expression of genes on chromosomes 13 and 18 might cause the abnormalities cited above.

We are therefore investigating the over-expression of candidate genes on chromosomes 13 and 18 in total RNA from various tissues (placenta, heart, lung, muscle, intestine, kidney, liver, spinal cord and brain) from trisomic fetuses and comparing the expression to normal age matched fetal tissues using northern blotting. Candidate genes being studied from chromosome 13 include collagen IV, retinoblatoma susceptibility anti-oncogene and esterase D. Esterase D is expressed in a range of tissues, but most strongly in liver and kidney and so is thought to play a role in detoxification. Candidate genes from chromosome 18 that are being studied include myelin basic protein (MBP), transthyretin (prealbumin) and laminin. Expression of MBP is seen in brain and spinal cord from the mid-fetal period onwards. There is thought to be an association between over-expression of MBP and mental retardation in trisomy 18.

To date expression has been observed in normal fetal tissues for transthyretin from chromosome 18 and esterase D and collagen IV from chromosome 13. Over-expression has been shown in trisomy 13 tissues for esterase D. All other candidate genes are under study.

We are also looking at the parental origin of the extra chromosome 13 or 18 and aim to determine if there is an association between methylation and expression of the candidate genes under study.

U 603 RECOMBINATION-MEDIATED ERCC5 DNA REPAIR GENE CORRECTION OF MUTANT RODENT CELLS,
Mark A. MacInnes, John S. Mudgett and Min Sung Park, Life Sciences Division, Los Alamos National Laboratory, Los Alamos NM 87545

Nucleotide excision repair proteins act to excise a wide variety of DNA lesions from chromatin. We have isolated functional cosmid clones of the human and mouse DNA excision repair genes *ERCC5*, via cosmid-mediated complementation of hamster UV sensitive mutant UV135 and of mouse Q31 cells. Northern blots indicate a size of ERCC5 mRNA for both human and mouse of 4.6 kb. Human and mouse pCD2 cDNA libraries have been screened and partial size inserts isolated and sequenced. However, in only one library screened by PCR [λgt11 library: a gift of D. Chen, Harvard University] was the predicted full length insert isolated. cDNA clones from the Okayama pCD2 libraries have been tested for correction of CHO UV-135 cells. All cDNAs were inactive for complementation of the UV sensitivity of UV135 cells alone. However, when transfected with cosmid DNA containing only the 5' end of ERCC5, cosmid-cDNA recombination occurred efficiently giving UV repair correction. The target of homologous recombination between cosmid and partial cDNA occurred within a single 1300 bp exon as demonstrated by genomic PCR amplification of the junction sequences. We will also present the deduced amino acid sequences of the ERCC5 genes and extent of evolutionary conservation with yeast Rad genes. [This work was supported by a Dept. of Energy contract, and an Alexander Hollaender Fellowship to JSM].

U 604 POSSIBLE EVIDENCE FOR GENOMIC IMPRINTING IN PRIMARY HAEMATOLOGICAL DISORDERS ASSOCIATED WITH MONOSOMY 7

Mitchell, C.D., Katz, F.E., Webb, D., Gibbons, B., Reeves, B., McMahon, C., Chessells, J.M., Leukaemia Research Fund Department of Haematology and Oncology and Department of Genetics, and ICRF Molecular Oncology Laboratory, St Bartholomews Hospital, London, U.K.

Monosomy or deletion of chromosome 7 is a frequent finding in both de novo and secondary acute myeloid leukaemia (AML) and myelodysplastic syndromes. Based on analysis of deletions of chromosome 7 in such patients, it has been suggested that there is a critical region of the chromosome lying within bands q21-31. We have examined bone marrow and peripheral blood samples from 10 patients with MDS, AML and biphenotypic acute leukaemia who had monomy for or rearrangement of chromosome 7, seeking evidence of non-random allele loss that might suggest the presence of imprinted genes on the chromosome.

Bone marrow cells from one patient with the infant monosomy 7 syndrome had loss of maternal alleles as did two patients with biphenotypic leukaemia. Five out of five patients with MDS and both patients with de novo AML had loss of paternal alleles. ONe of the latter patients had a del(7)(q31q36) rather than monosomy 7. These findings suggest that imprinting of a gene(s) on chromosome 7, within the bands q31-q36, may be of importance in MDS and AML.

Despite the reported increased incidence of AML amongst relatives of patients with cystic fibrosis (CF) the gene for which lies in chromosome region 7q31, none of the patients nor parents studied here appeared to be carriers of the most common gene mutation seen in patients with CF, the delta F508.

U 605 TOPOISOMERASE I MUTATIONS IN FANCONI ANEMIA CELLS

Robb E. Moses and Hiroshi Saito
Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland OR 97201

Fanconi Anemia(FA) is an autosomal recessive disorder exhibiting various congenital defects including short stature, progressive hypoplastic pancytopenia, and dark skin pigmentation. The patients develop leukemia and malignant neoplasms with high frequency. Cells from many FA patients show elevated levels of chromosome breakage and increased sensitivity to DNA cross-linking agents such as mitomycin C (MMC), suggesting a deficient DNA cross-linking repair mechanism may be the primary defect in FA. Recent linkage analysis demonstrated that FA in some families shows linkage to chromosome 20q (Mann et al., Genomics 9:329-337 [1991]). The topoisomerase I gene (Top I) has been mapped to human chromosome 20q (Juan et al., Proc. Natl. Acad. Sci. USA 85:8910-8913 [1988]). DNA topoisomerase I is a protein which may participate in DNA repair and genome stability. These observations prompted us to study Top I as a FA candidate gene. First, FA cells and control cells were exposed to MMC with or without camptothecin (CPT), a topoisomerase I inhibitor. The cells did not show any increased sensitivity to killing by MMC with CPT, suggesting that the topoisomerase I is not involved in the MMC DNA repair system. However, FA cells showed increased sensitivity to CPT in comparison with control cells, raising the possibility of altered topoisomerase I in FA cells. Top I cDNA was amplified by polymerase chain reaction from reverse transcribed cDNA from RNA isolated from seven different FA cell strains. Heteroduplex formation was performed between Top I cDNA from FA subjects and ^{32}P end-labeled Top I cDNA from a control subject. The heteroduplex was then analyzed by chemical cleavage to detect any base mismatch between the FA and control Top I cDNA. By this mutation scanning analysis, mutations were found in the gene from three different FA cell lines. DNA sequencing analysis confirmed these three mutations and showed that all of them were point mutations causing an amino acid change in the topoisomerase I protein. A two base pair deletion causing a frame shift was also found in one of the three FA Top I cDNAs.

This work was supported by a grant from the Medical Research Foundation of Oregon.

U 606 DYSTROPHIN IS EXPRESSED IN THE HUMAN RETINA AND MAY BE REQUIRED FOR NORMAL FUNCTION.

Peter N. Ray[1], Dennis E. Bulman[1], Lawrence E. Becker[1], Ronald G. Worton[1], Berkley R. Powell[2], Richard G. Weleber[2], De-Ann M. Pillers[2]
[1]Hospital for Sick Children, Toronto, Canada, M5G 1X8. [2]Oregon Health Sciences University, Portland OR. 97201

Dystrophin, the product of the Duchenne muscular dystrophy (DMD) gene is found predominantly in muscle but is also found, in lesser amounts, in a number of non-muscle tissues including neurons and glia. Using 3 dystrophin specific antisera that were raised against different domains of the protein, we examined normal human retina for the presence of dystrophin. These studies indicated that the DMD gene is expressed in the retina and that dystrophin is localized to the outer plexiform layer. Western blot analysis revealed that retinal dystrophin is present in two isoforms, both of which are smaller than the muscle isoform. These isoforms of dystrophin are the result of unique alternative splicing and have not been observed in other tissues.

In previous studies on a boy (Oregon-JR) with glycerol kinase deficiency, congenital adrenal hypoplasia and Duchenne muscular dystrophy, we found an abnormal electroretinogram (ERG) that was virtually identical to that observed in patients with Åland Island eye disease (AIED) and incomplete congenital stationary night blindness (CSNB1). Whereas AIED was mapped proximal to the DMD gene on the short arm of the X chromosome, the deletion in this patient removed approximately 1/3 of the DMD gene and extended distally on the X-chromosome.

To determine if the lesion in the DMD gene contributed to the retinal dysfunction in this patient we performed electroretinograms on several Duchenne and Becker muscular dystrophy patients with deletions in the DMD gene. All patients had negative ERGs characterized by the absence of the 'b'-wave similar to those seen in patients with CSNB1 and Åland Island eye disease. Dystrophin appears therefore to be necessary for the cellular processes that result in the generation of the ERG 'b'-wave.

U 607 HUMAN FIBROBLAST cDNA-MEDIATED TRANSDUCTION OF X-RAY RESISTANCE TO X-RAY SENSITIVE CHO CELLS.

A.J.Varghese and G.F.Whitmore, Ontario Cancer Institute, Toronto, Ontario, M4X 1K9 Canada. Fibroblast cells from A-T patients are hypersensitive to X-rays and antitumour drugs bleomycin and etoposide and have the same sensitivity as normal cells to UV light and mitomycin C. XRS-T1 is an X-ray sensitive mutant of CHO cells isolated in our laboratory and exhibits properties similar to those of A-T cells. The hypersensitivity of A-T cells and XRS-T1 cells to DNA-damaging agents that produce a particular type of damage is probably due to a gene-product deficiency. We have tried to correct this defect in XRS-T1 cells by DNA transfer. From XRS-T1 cells, after transfection with a human fibroblast cDNA library (pcD2-cDNA-neo), we have isolated a geneticin-resistant and X-ray-resistant clone(J-15). From a lambda phage library prepared from the total cellular DNA from J-15 cells, using pcD2-neo for plaque hybridization, individual phages were picked and were used to transfect XRS-T1 cells. Geneticin-resistant transformants were tested for X-ray sensitivity.Transformants from two phages were X-ray resistant. BamH1 cleavage of these phages revealed the presence of a common fragment and is most probably the cDNA capable of transferring radiation resistance to radiation sensitive cells (a repair gene?). Experiments are in progress to clone and sequence the cDNA fragment.

U 608 TESTES AND SOMATIC *mXRCC1* DNA REPAIR GENE EXPRESSION, Christi A. Walter[1], Jianwei Lu[1], Zi-Qiang Zhou[1], Mukesh Bhakta[1] and Larry H. Thompson[2], [1]Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284, [2]Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore CA 94550

The expression patterns of specific DNA repair genes during the mammalian life span remain largely unknown. We are developing mouse model systems to study a mammalian DNA repair gene, *XRCC1*. The human *XRCC1* gene was shown to be involved in resistance to ionizing radiation and to play a role in DNA strand break repair by complementing the DNA repair deficient Chinese hamster ovary cell line EM9 (Thompson et al., 1990, *Molec. Cell. Biol.* 10:6160-6171). The *XRCC1* gene product is a candidate for providing protective DNA repair activity throughout the life span of an animal. We have begun to systematically analyze expression of murine *XRCC1*. Results from Northern blot analyses and RNAase protection assays, performed with RNA isolated from select mouse tissues, reveal expression of murine *XRCC1* is high in testes relative to other tissues and to newborn pups. Testes *mXRCC1* expression is approximately 10-fold higher than in heart or newborn pups, 4-fold higher than brain and 3-fold higher than liver when standardized against actin expression. Additional analysis indicates there may be differences in the level of expression during sperm maturation. The 5' flanking region of the mouse *XRCC1* gene is being subcloned and analyzed to identify cis-acting DNA regulatory elements involved in directing the tissue-specific and developmental-specfic expression patterns of this gene.

This work was aided by Reproductive Hazards in the Workplace, Home, Community and Environment Research Grant No. 15-167 from the March of Dimes Birth Defects Foundation, AG00165 and AG06872 from the NIH. A portion of this work was done under the auspices of the U.S. DOE by LLNL under contract number W-7405-ENG-48.

U 609 GENERATION OF MOUSE MODELS FOR HUMAN REPAIR SYNDROMES, G. Weeda, C. Troelstra, M. Hoogeveen-Westerveld, I. Donker, D. Bootsma, and J.H.J. Hoeijmakers, MGC-Dept. of Cell Biology and Genetics, Erasmus University. P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Patients suffering from the rare, autosomal, recessive repair disorders xeroderma pigmentosum (XP) and Cockayne Syndrome (CS) exhibit severe sun (UV)-sensitivity, frequently neurologic degeneration and -in the case of XP- cutaneous abnormalities and predisposition to skin cancer. The molecular defect in these genetically heterogeneous diseases resides in the complex nucleotide excision repair system. Using excision deficient (UV-sensitive) rodent mutant cell lines of different complementation groups we have isolated several complementing human repair genes.

The *ERCC-1* gene specifically corrects rodent group 1 mutants (that are highly sensitive to UV and to DNA crosslinking agents) and appears not involved in any of the XP and CS complementation groups. The *ERCC-3* gene, encoding a putative DNA helicase corrects rodent group 3 mutants and cells from patients of the very rare combined XP/CS group B. Different mutations in the gene have been identified in XP-B patients exhibiting clinical variation particularly with respects to carcinogenesis. The homologous repair gene in yeast has a vital function. *ERCC-6*, specifying also a presumed helicase, corrects rodent group 6 mutants and cells from CS group B patients. The protein appears to be specifically involved in the preferential repair of active genes. For gene targetting in mouse embryonal stem cells replacement vectors were constructed with *neo* and *hyg* expression cassettes inserted in coding exons to create null alleles. To circumvent the problem of possible lethality, also targetting constructs were made in which subtle mutations found in patients are mimicked. It is expected that the generation of repair deficient mice will improve our understanding of mutagenesis, carcinogenesis, aging and neurodegeneration. Furthermore such mice can be used as a valuable sensitive animal model for testing the mutagenic and potential carcinogenic agents.

Late Abstracts

ALTERED STRUCTURE AND METHYLATION OF PROTO-ONCOGENE AND TUMOUR SUPPRESSOR LOCI IN HUMAN PANCREATIC CARCINOMAS. Jean-Luc Dionne, Fai Lee and Rémy A. Aubin, Health and Welfare Canada, Life Sciences Division, Sir F.G. Banting Res. Ctr, Tunney's Pasture, Ottawa, Ontario K1A OL2

Malignant tumours of the exocrine pancreas are ranked as the fourth leading cause of cancer-related death in males in North America. This high mortality rate reflects the aggressive nature of the disease which is often metastatic at the time of diagnosis. Since developing pancreatic cancers produce vague and non-specific symptoms, potential early warning signs are often ignored by both patient and physician. Poor prognosis, therefore, stems from our inability to detect and stage malignancies at a period when they may be surgically curable. It is within this practical framework that we have initiated efforts to uncover genetic lesions which may play a reproducible and predictable role in the initiation and/or progression of pancreatic tumours. Allelic profiles of the *c-Ha-ras*, *c-Ki-ras*, *N-ras*, *c-myc*, *c-erbA*, *EGFR*, *Her2/neu*, *c-ets* and *p53* loci were studied in 9 human pancreatic carcinoma cell lines. Southern hybridization analysis revealed allelic loss at the *c-ets* and *p53* loci. In addition, one tumour line appeared to have suffered a rearrangement of the *p53* gene. Rare alleles of the *c-Ha-ras* gene were not encountered and the *Her2/neu* gene was found to be amplified. Finally, anomalous patterns of DNA methylation were observed within the regulatory regions of the *c-Ha-ras* and *c-myc* genes.

RETROVIRAL INSERTIONS DOWNSTREAM OF THE GENE FOR RNA-BINDING A1 IN ERYTHROLEUKEMIA CELLS INDUCED BY FRIEND LEUKEMIA VIRUS. Ben-David[1], Y., Chabot[2], B., De Koven[1], A. and Bernstein[3], A. [1]Division of Cancer Research, Sunnybrook Health Science Centre, Toronto, Ontario, Canada, [2]Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada and [3]Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

The multistage erythroleukemia induced by the various strains of Friend leukemia virus involved inactivation of the *p53* tumor suppressor gene and frequent activation of either the *Spi-1/PU.1* or *Fli-1* genes. Both *Fli-1* and *Spi-1* are members of the *ets* oncogene family of transcription factors and are activated as a result of proviral integration events in a majority of erythroleukemia cell lines induced by either Friend murine leukemia virus (F-MuLV) or Friend spleen focus-forming virus (SFFV), respectively. In this report, we have identified a novel common integration site, designated *Fli-2* (Friend leukemia integration-2), from an erythroleukemia cell line induced by F-MuLV. Rearrangements at the *Fli-2* locus were found in 2/11 erythroleukemia cell lines independently induced by F-MuLV and 1/19 erythroid cell clones derived from spleens of mice infected with the polycythemia or anemia strains of Friend virus (FV-P abd FV-A respectively). A cDNA corresponding to a transcript originating from genomic DNA adjacent to *Fli-2* was isolated using a junction fragment 5' to the site of the proviral integration site. The deduced amino acid sequence of this cDNA is identical to the human *hnRNP* A1 gene, a member of the gene family of RNA binding proteins involved in RNA slicing. In one erythroleukemia cell line, A1 expression is undetectable as a result of F-MuLV integration in one allele and loss of the other allele. These results provide the first evidence that perturbations in the regulation of RNA splicing may contribute to neoplastic transformation.

TWO HOT SPOTS OF RECOMBINATION IN THE DMD GENE, Claudine L. Oudet, André Hanauer, Paul Clemens, Jeffrey Chamberlain, Thomas C. Caskey and Jean-Louis Mandel, CNRS-LGME, and INSERM-U 184, Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg France, and Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030.

Among the mutations observed in DMD/BMD patients, 70% are deletions. Multiplex PCR reactions (Chamberlain et al. 1988, 1990; Beggs et al., 1990) detect almost all deletions in affected males. However, intragenic and flanking markers are still commonly used for prenatal diagnosis and genetic counseling. In DMD patients, as well as in healthy control populations, the DMD gene has been shown to recombine with a frequency which rises to 12%(Chen et al., 1989; Abbs et al., 1990; Oudet et al., 1991). Chen et al. (1989) detected a hot spot of recombinations between DXS84 and DXS206. Abbs et al. hypothesized the existence of another hot spot in the P20 region of the gene, since it is highly prone to deletions (Koenig et al., 1989, Den Dunnen et al., 1989). Using highly polymorphic microsatellite intragenic markers recently developed by several authors (Beggs et al., 1990; Clemens et al., 1991; Feener et al., 1991; Oudet et al., 1990, 1991; Powell et al., 1991, Roberts et al., 1989, 1990), we have typed the CEPH families panel . There is a major hot spot of recombinations between the markers STR44 and STR50 (Clemens et al., 1991), i.e. between the exons 44 and 51. Within the hot spot, a peak is located between the exons 44 and 45, in the large intron 44. A second, however minor, hot spot occured between the marker XJ (in the large intron 7) and the 5'end of the DMD gene. When the distribution of the recombination events in the gene of healthy individuals was superimposed with the distribution of the deletions in DMD/BMD patients, there was an obvious correlation between both phenomena . Our results should improve the linkage analysis applied to carrier detection and prenatal diagnosis.