BIOTECHNOLOGY AND HUMAN GENETIC PREDISPOSITION TO DISEASE

Organizers: Charles Cantor, C. Thomas Caskey, Leroy Hood, Daphne Kamely and Gilbert Omenn

March 27-April 3, 1989

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Detection

K 001 EVOLUTIONARY ANALYSIS OF POSITION 57 IN THE HLA-DQ AND DRß CHAINS IN PRIMATES INDICATES IT IS A BALANCED POLYMORPHISM WITH POSSIBLE CONFORMATIONAL IMPORTANCE. Ulf B. Gyllensten[1,2], Deval A. Lashkari[1], Ines Ezcurra[1] and Henry A. Erlich[1]. 1. Department of Human Genetics, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608, USA. 2. Department of Medical Genetics, Biomedical Center, University of Uppsala, Box 589, S-75123 Uppsala, Sweden.
Genetic susceptibility to several autoimmune diseases e.g. insulin dependent diabetes mellitus (IDDM) and Pemphigus vulgaris (PV) has been shown to be correlated with a neutral amino acid (Ala, Val, Ser) and resistance with a charged amino acid (Asp) at position 57 in the HLA-DQß chain. The functional significance of position 57 is further suggested by the conservation of this charge polymorphism in all human class II ß chains. We have used PCR to examine the nature of the polymorphism in a number of primates including chimpanzee, gorilla, baboon, rhesus, langur and cebus. The polymorphism at this position is highly conserved at all loci examined (DQß, DRßI and DRßIII) in all the primates, with a very restricted set of residues accepted (Asp, Ser, Ala or Val). The evolutionary maintenance of a balanced polymorphism in all ß-chains over at least 10-20 million years suggests that it may be of funtional importance, by possibly affecting the structure of the class II molecule.

K 002 FLUOROGENIC REPORTER GENE ANALYSIS AND SORTING OF VIABLE CELLS, Leonard A. Herzenberg, Dept. of Genetics, Stanford University, Stanford, CA 94305 The FACS/GAL assay for E. coli lacZ gene expression in eucaryotic cells (1) utilizes the Fluorescence Activated Cell Sorter (FACS) to measure ß-galactosidase (ß-gal) activity in individual viable cells. With this technique we have successfully studied a variety of gene regulatory, gene context and gene detection problems in mammalian cells. I will describe other interesting uses of FACS/GAL, including variation in expression levels of lacZ in clones where various promoter/enhancer elements are stably integrated via retroviral infection or transfection; identification of differentiation dependent enhancers in a murine pre-B cell line; rapid detection of immunoglobulin recombinase activity; analysis of activation in a human T cell line; and identification of genes whose products have different intracellular locations.

The FACS and flow cytometry have traditionally been used to detect and count hematopoietic and other cells bearing different surface antigens, to sort rare variants of surface antigens, to analyze DNA and cell cycle position, and to sort chromosomes. Now, FACS/GAL combined with multiparameter analysis permits identification of differentiated cell types expressing galactosidase in various molecular contexts in chimaeric and transgenic mice.

1) Proc. Natl. Acad. Sci. (1988) 85: 2603

K 003 MEASURING RESPONSES OF CELL LINES TO DRUGS AND TOXINS WITH AN ULTRASENSITIVE SILICON SYSTEM, J. Wallace Parce, Karen M. Kercso,and John C. Owicki, Molecular Devices Corporation, 4700 Bohannon Drive, Menlo Park, CA 94025

A variety of cell affecting agents may be detected based on measurement of the metabolic rate of cells repetitively and with a high degree of precision. These cell affecting agents may be toxins, nutrients, or ligands for specific cell receptors to name just a few. Using the light addressable potentiometric sensor (LAPS) technology (Hafeman et.al.) we have constructed a micro flow chamber in which living cells can be maintained for long periods of time. By detection of the rate of medium acidification during intermittent interruption of the flow of medium over the cells, the metabolic rate of the cells can be determined approximately once every three minutes. Precise measurement of the metabolic rate of the same group of cells before and after introduction of the cell affecting agent in the flow stream allows for detection of these agents at physiologically relevant concentrations.

Hafeman, D.G., Parce, J.W., and McConnell, H.M. Light-Addressable Potentiometric Sensor for Biochemical Systems. Science 240, 1182-1185 (1988).

K 004 FLUORESCENT DETECTION OF HUMAN CHROMOSOME-SPECIFIC DNA PROBE SETS: DIAGNOSTIC AND BIOLOGICAL APPLICATIONS, D.C. Ward, P. Lichter, T. Cremer, C.C. Tang, M. Ferguson and L. Manuelidis, Department of Human Genetics and Section of Neuropathology, Yale University School of Medicine, New Haven, CT 06510. A method of *in situ* hybridization for visualizing individual human chromosomes from pter to qter fluorometrically has been developed and applied to the detection of normal and aberrant chromosomes both in metaphase spreads and interphase cells. Numerical changes, deletions and chromosomal translocations were rapidly delineated in oligodendroglioma and glioblastoma cell lines which possess very complex and highly aneuploid karyotypes. Specific translocations diagnostic of Burkitts Lymphoma and Chronic Myeloblastoid Leukemia were also identified. A trisomic chromosome 21 karyotype, diagnostic of Down syndrome, was also readily detected using either a complete pool of insert DNA from a chromosome 21 recombinant DNA library or plasmid clones containing up to 94 kilobases of single copy DNA from band q22.3 of chromosome 21. Such *in situ* hybridization strategies provide new approaches for the prenatal diagnosis of genetic diseases and the definition of abnormal chromosomes in tumor cell populations. Laser-scanning confocal fluorescence microscopy was used to aid the 3-D visualization of chromosomal domains in interphase cells and to provide quanitative fluorescence data. By labeling DNA probes directly with different fluorochromes or with different reporter molecules that are uniquely detected by fluorochrome labeled reporter-specific proteins, it is possible to visualize multiple probe sets simultaneously. Fluorescent *in situ* hybridization procedures have also been used to rapidly map the chromosomal *location* of multiple cosmid or phage clones containing human DNA inserts, to localize genes in interphase nuclei and to analyze intranuclear chromosome topography.

Biotechnology and Human Genetic Predisposition to Disease

Cancer: Oncogenes, Anti-Oncogenes and Ecogenetics

K 005 CONSTRUCTING A MOUSE MODEL FOR RETINOBLASTOMA, Tyler Jacks, Amin Fazeli, Rene Bernards, En Li, Rudolf Jaenisch, and Robert A. Weinberg, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142

Retinoblastoma (RB) is the best studied of a growing number of human cancers believed to arrise, at least in part, due to the loss of function of a negative (or recessive) oncogene. Individuals who inherit one defective copy of the *rb* negative oncogene have a 90% likelihood of developing RB (usually at multiple sites in both eyes) via mutation or loss of their one functional *rb* allele. These familial RB patients are also predisposed to a number of other tumor types, particularly osteosarcoma. RB can also occur in individuals with no family history of the disease through the sequential loss of the two *rb* alleles in a given cell. Characterization of the *rb* gene, its protein product, and tumor-associated mutations have been made possible by the cloning of a cDNA copy of *rb* mRNA.

In order to assess what additional genetic or environmental factors might affect development of RB, we are attempting to construct a model for this disease in the mouse. Our strategy is to first disrupt one allele of the *rb* gene in mouse embryonal stem (ES) cells in culture, followed by the introduction of these *rb+/rb-* cells into normal blastocysts, and, finally, breeding of the resulting chimeric animals to derive mouse strains that are constitutionally hemizygous for *rb*. cDNA cloning and sequencing of the murine homologue of the human *rb* gene has shown approximately 90% identity between the two predicted amino acid sequences. The gene is expressed in a wide range of mouse tissues, including ES cells. We have used this cDNA to clone fragments of the mouse *rb* gene into which have been engineered several deleterious mutations. We are currently using the polymerase chain reaction (PCR) method to screen for ES cells that have transferred these mutant *rb* sequences into one chromosomal copy of the *rb* gene by homologous recombination. We have shown that PCR will allow detection of one homologous recombination event in 10^5 cells.

K 006 ECOGENETICS OF CHEMICAL CARCINOGENS, Gilbert S. Omenn, Departments of Environmental Health and Medicine, and Charles A. Dana Program in Genetics and Environmental Health, University of Washington, Seattle, WA, 98195.

The potential carcinogenicity of exogenous chemicals depends not only on the intrinsic properties and dose of the chemical, but also on host responses. To become carcinogenic, many chemicals must be activated enzymatically, usually by cytochrome P450 monooxygenases, to genotoxic electrophilic intermediates. Other enzyme systems detoxify carcinogenic chemicals by hydrolysis of epoxides, conjugation with glutathione, or acetylation. My colleagues and I are interested in the genetically-determined variation in these molecular biotransformations and the potential significance of common variants (ecogenetic polymorphisms) in humans for differential risk from similar environmental exposures.

Investigations with sequence probes and/or enzymatic assays for various glutathione S-transferases, for microsomal epoxide hydrolase, and for various P450s, especially the P450 determining the debrisoquine polymorphism, will be presented. In addition, the importance of the N-acetyl transferase polymorphism for effects of beta-naphthylamine and benzidines as urinary bladder carcinogens will be discussed.

We may predict that analogous polymorphic variation in genes influencing oncogene activation, tumor suppression, and other host responses will be found as the molecular mechanisms for these phenomena are elucidated.

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Developmental Defects

K 007 THE MURINE W LOCUS: A GERM-LINE MUTATION IN A MAMMALIAN PROTO-ONCOGENE, Alan Bernstein, Karl Nocka, Patrice Dubreuil, Sadhan Majumder, Alastair Reith, Prabir Ray, Benoit Chabot, and Peter Besmer, Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, Toronto, Canada, M5G 1X5, and Molecular Biology Program, Sloan Kettering Institute, New York, NY. Mutations at the mouse W Locus can lead to pleiotropic developmental defects, including sterility, coat colour abnormalities, severe macrocytic anemia and mast cell deficiency. The cellular defects in all these lineages result from a cell autonomous, intrinsic defect that affects the proliferation and/or migration of cells early in embryogenesis and in adult life. In an attempt to understand the molecular basis of this classical mouse developmental mutant, we, and Geisler and Housman, have recently provided genetic and molecular evidence that the c-kit proto-oncogene is allelic with the W locus. DNA sequence and protein analysis has established that the c-kit gene is a member of a gene family of transmembrane growth factor receptors with protein tyrosine kinase activity that includes c-fms and the PDGF receptor. Consistent with the intrinsic nature of the cellular defects in W mutant mice, the c-kit proto-oncogene is expressed in those tissues and cell types affected by mutation at the W locus. In addition, expression of c-kit is markedly reduced in the severely anemic fetal livers derived from homozygous W/W mutant embryos. Further studies on the protein product of c-kit in W mutant animals and on the molecular basis of the mutant phenotypes observed in the many independent W mutations provides a unique opportunity to analyze the developmental consequences of germ-line mutations in a mammalian proto-oncogene.

K 008 ORGANIZATION, EXPRESSION, AND EVOLUTION OF A TYPE MAMMALIAN HOMEOBOX GENES, Frank H. Ruddle, Claudia Kappen and Klaus Schughart, Department of Biology, Yale University, New Haven, CT 06511

The A type mammalian homeobox genes show sequence similarities with *Drosophila* homeotic genes of the Antennapaedia and Bithorax complexes. As in *Drosophila*, mammalian genes are clustered and have an ordered arrangement on the chromosome colinear to transcript expression along the anterior/posterior body axis. The mammalian genes differ in that they exist as four clusters on different chromosomes. The organization of genes within a given cluster is very similar to the organization of genes in the other clusters. Both phyletic and claudistic analysis on the homeoboxes within clusters indicate that they have arisen in highly similar if not identical ways. This argues for the A type homeobox gene family having arisen by expansion of a primordial gene cluster followed in chordate lines of ascent by cluster multiplication mediated most probably by genome duplication. Cluster expansion is marked by rapid sequence divergence, whereas cluster multiplication is comparatively conservative. Implications of these results for chordate evolution will be discussed, as well as experimental systems which may prove useful in testing this hypothesis.

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K 009 THE SEX-DETERMINING FUNCTION OF THE HUMAN Y CHROMOSOME, Ansbert Schneider-Gadicke, Peggy Beer-Romero, Laura G. Brown, Elizabeth M.C. Fisher, Shiuh-Wen Luoh, Graeme Mardon, Elizabeth M. Simpson, Steven Swendeman and David C. Page.

We have found that whether a human embryo develops as a male or female is determined by the presence or absence of a very small portion of the Y chromosome, 140 kb interval 1A2. This sex-determining region carries a gene, *ZFY*, that might constitute the sex-determining switch. Curiously, there exists a closely related yet distinct gene, *ZFX*, on the human X chromosome. *ZFY* and *ZFX* show remarkably similar intron/exon organizations and exon DNA sequences. Very similar sequences exist on the Y and X chromosomes of a wide range of placental mammals and *ZFY/ZFX* possibly derived from a common ancestral gene prior to the radiation of placental mammals. Both genes encode zinc finger proteins with a highly acidic domain, suggesting that they activate transcription. The zinc finger domains are particularly similar, both encoding 13 fingers with a two finger repeat unit and 383 of 393 amino acid residues being identical. Thus, the ZFY and ZFX proteins may bind to and regulate the same genes. *ZFY* and *ZFX* are transcribed in a wide variety of XY and (in the case of *ZFX*) XX cells. Transcription of *ZFY* and *TFX* is not dosage compensated and *ZFX* escapes X-inactivation. We discuss implications of these findings for models postolating roles for both *ZFY* and *ZFX* in gonadal sex determination.

Mapping the Human Genome

K 010 THE CONSTRUCTION OF ORDERED GENOMIC LIBRARIES USING "OLIGOFINGERPRINTING"

Hans Lehrach, Alister Craig, Dean Nitezic, Jörg Hoheisel, Greg Lennon, Tony Monaco, Günther Zehetner. Imperial Cancer Research Fund, Lincoln's Inn Fields, PO Box 123, London WC2A 3PX, UK.

To reduce the effort required to establish the physical maps based on ordered clone libraries from mammalian chromosomes or genomes we have started to develop an alternative approach to the gel electrophoresis fingerprinting procedures used very successfully in the analysis of smaller genomes, based on the hybridisation of short oligonucleotides to DNA spots generated either from in-situ lysis of cosmid, lambda or potentially also YAC clones or from e.g. spoted phage lysates or DNA samples. Experimental result, analysis and evaluation are very similar to the results in genetic linkage experiments. To test this approach, the completely sequences genome of HSV-1 has been used as test case. 400 cosmids (100 genome equivalents) have been picked, spotted at high density using a robotic device, and hybridised to a set of oligonucleotides picked from the sequence, allowing us to optimise the design of oligonucleotide probes and hybridisation conditions. As a next step cosmid and phage libraries have been prepared from the genome of S. Pombe (50 genome equivalents) and from DNA from flow sorted human X chromosomes (15 chromosome equivalents), and partly spotted as high density replicas of the primary libraries stored in microtiterplates. Work on the construction of a YAC library of the human X chromosome has started.

K 011 **THE USE OF MOBILITY-SHIFTING NUCLEOTIDE ANALOGS TO DETECT DNA MUTATIONS AND POLYMORPHISMS**, Kenneth J. Livak and J. Stephen Kornher,
Central Research & Development Dept., E. I. du Pont de Nemours & Co., Wilmington, DE 19880
We have devised a new, general procedure that will detect single nucleotide polymorphisms at any site in a DNA fragment, not just those affecting restriction sites. The method exploits the fact that the incorporation of certain nucleotide analogs into DNA causes a detectable shift in electrophoretic mobility. The method involves using a DNA polymerase to synthesize DNA strands of defined length, replacing one of the four dNTPs with a mobility-shifting analog. DNAs that are the same length but differ in the number of analog molecules per strand will exhibit different mobilities on a sequencing gel. This provides a rapid assay for distinguishing DNAs that may be identical in length but differ in base composition. To demonstrate this technique we have analyzed a nonsense mutation found in the gene encoding the human insulin receptor [Kadowaki *et al.*, *Science* **240**:787-790 (1988)]. Using PCR, a 140-bp segment containing the mutational site was amplified in genomic DNA isolated from a patient heterozygous for this mutation and from an homozygous normal individual. Each of the amplified DNAs was analyzed by primer extension using biotin-11-dUTP in place of TTP. On a sequencing gel, the primer extension product from the mutant allele migrates at a position one nucleotide slower than the product from the normal allele because the mutant product contains one more biotin-11-deoxyuridine residue than the normal product. This distinguishes the heterozygote from the homozygote. Similar analyses have been performed with an analog of dCTP.

K 012 MAKING RESTRICTION MAPS OF WHOLE CHROMOSOMES, Cassandra L. Smith,
Departments of Microbiology and Psychiatry, College of Physicians and Surgeons, Columbia University, New York, NY 10032
Large DNA technology allows the construction of low resolution restriction maps from virtually any organism. The difficulty of the task scales with the size of the region or genome that is to be mapped. Completing such maps are the major challenge. For small genomes, making a catalogue of megabase restriction fragments by hybridization experiments with single copy clones will provide almost completed maps. For larger genomes, a combination of strategies must be employed. A variety of strategies were developed and used for completing the maps of *E. coli* and *S. pombe* which are 5 and 15 Mb, respectively. These strategies are now being used to construct a complete restriction map of human chromosome 21 (50 Mb in size). These strategies include the use of linking clones, telomere clones, partial digests and the exploitation of naturally occurring genomic polymorphisms.

K 013 THE GENOME OF CAENORHABDITIS ELEGANS, Robert H. Waterston[1], Humaira Ameer[1], Alan Coulson[2], Yuji Kohara[2] and John Sulston[2], [1]Department of Genetics, Washington University, St. Louis, MO 63110 and [2]MRC Laboratory of Molecular Biology, Cambridge, England CB2 2QH.

We are developing a physical map of this 100 Mb nematode genome through cloned fragments recovered in cosmid and YAC (yeast artificial chromosome) vectors. With the cooperation of many researchers the physical map is being aligned with the genetic map and the result represents a powerful tool for molecular genetics.

The initial stage of the project involved characterization of cosmid clones using a fingerprinting technique that produced a pattern of restriction fragments from each clone. Overlaps between cosmids were detected by computer comparison of the patterns, allowing the alignment of cosmids into groups of overlapping clones, called contigs. After analysis of more than 17000 clones, about 700 contigs (down from a high of 940) had been obtained, and about 90% of the genome was contained in them. Further progress was limited by the scarcity of the missing segments in the cosmid libraries, and indeed there was evidence that certain regions of the genome were completely lacking from the libraries.

We have therefore begun to use YAC libraries to link the existing contigs. Hybridization is used to recognize sequences in common between randomly selected YACs and a canonical set of cosmids representing the pre-existing contigs. Hybridization of a single YAC to two contigs indicates a possible adjacency of the contigs and, provided the fit meets logical constraints, a join is made. Through these efforts which have involved both walking and random clone analysis we have now have 250 contigs. Some 60% of the genome, represented in about 40 of the larger contigs, is aligned with the genetic map.

While the mapping operation continues, the existing contigs (which range in size to >5Mb) are in constant demand for molecular genetic projects in various *Caenorhabditis elegans* laboratories. As well as providing material for the cloning of genes, the contigs thereby become increasingly aligned with the genetic map, so that by the time linkage is complete there will already be numerous points of correspondence between the two maps.

Supported in part by USPHS and MDA grants to R.H.W.

K 014 RECENT ADVANCES IN MAPPING THE HUMAN GENOME: RFLP MAPPING, Raymond L. White, Howard Hughes Medical Institute, Salt Lake City, UT 84132

Preliminary genetic linkage maps of markers for all the human chromosomes are virtually complete; most genetic defects that are segregating in families should now be amenable to linkage studies that will localize them to a specific chromosomal region. This achievement is the result of a massive program undertaken a few years ago in our laboratory and others to develop new markers based on restriction fragment length polymorphisms in the human genome, and to design computer programs capable of ordering them into maps by analyzing linkages among several marker loci at a time, in a large panel of reference families. Hundreds of markers, many of them highly efficient for disease mapping because they reflect loci (VNTRs) with a high probability of heterozygosity within families being tested, have been placed on chromosome maps. Underway now is an attempt to cover each chromosome with markers spaced only about 1 megabase apart, to make the initial localization of disease-causing genes more efficient and to narrow regions that must be searched to identify the respective molecular defects. Even the initial localization of some diseases will require markers that are this closely spaced. We already have fine-structure linkage maps, achieved by developing a large number of markers targeted to a particular region, in the vicinity of some genes of particular interest, for example the loci harboring the mutations responsible for adenomatous polyposis of the colon (on chromosome 5) and neurofibromatosis type 1 (on chromosome 17). This degree of resolution gives us markers, flanking the gene of interest, that describe a region small enough for us to exploit a number of techniques to search for the gene itself. As the molecular basis of most genetic diseases and predispositions is unknown, linkage mapping represents an important approach to identification and subsequent cloning of a gene responsible for a particular disorder.

DNA Sequencing

K 015 DEVELOPMENT OF MACHINES FOR DNA SEQUENCING STAGES AND THEIR ORGANIZATION INTO A LINE SYSTEM. Eiich SOEDA and Akiyoshi WADA, Riken Gene Bank, The Institute of Physical and Chemical Research, 3-1-1 Koyadai Tsukuba Science City 305, and Department of Physics, Faculty of Science, University of Tokyo, Tokyo 113, Japan.

Shotgun strategy is currently regarded as the rapidest sequencing approach and being most applicable to an automated line system which makes it possible to produce massive raw sequencing data. It consists virtually of seven successive stages . Of these, the latter halves were the rate limited against rapid sequencing. We have developed the machines and equipments involved in the entire stages of sequencing since 1981 under the financial support from the Agency of Science and Technology of Japan. At first, our effort has been made to substitute the hand-manipulations at the latter halves by automated machines using existing technologies. Some of them have been completed and a feasibility study has been examined using a human cosmid clone in order to the maximum speed as well as the largest unit of sequencing.

Introducing of the automated machines led rapid and massive production of sequencing raw date. However, it was found that the presence of Alu repeats characteristic of the human genomic DNA interrupted against completion of the human cosmid sequencing, suggesting and that compilation of sequence data management is important as well as massive production of the sequence data.

K 016 APPROACHES TO SOLID-PHASE DNA SEQUENCING. Mathias Uhlén, Tomas Moks and Thomas Hultman. Department of Biochemistry, Royal Institute of Technology, S-10044 Stockholm, Sweden. New approaches have been developed using solid-phase DNA sequencing. Biotin is selectively introduced into one of the strands of the double stranded DNA template and this biotin is subsequently used for directed immobilization to magnetic beads coated with streptavidin. Sequencing based on the Sanger method using isotope (P-32 and S-35) or fluorescence labels is performed after selective elution of the non-biotinylated strand with alkali or formamide. This concept has been used for sequencing of double stranded template either in the form of plasmid DNA or as amplified material from polymerase chain reaction (PCR). New sequencing strategies will be described to facilitate sequencing of large DNA inserts, taking advantage of the solid phase technology. In addition, a work station for automated plasmid purification from overnight *E.coli* cultures has been developed for preparation of large numbers of plasmid DNA suitable as template for solid-phase sequencing.

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Heart Disease

K 017 GENETIC BASIS OF CORONARY HEART DISEASE, Jan L. Breslow, Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York, NY 10021

Coronary heart disease (CHD) risk is directly proportional to LDL and inversely proportional to HDL cholesterol levels. Intense effort is being expended to identify the loci that harbor common variations that explain differences in LDL and HDL levels and atherosclerosis susceptibility in the population. LDL particles have one molecule of apo B-100 on their surface. This protein is the ligand recognized by LDL receptors, which clear these particles from the circulation. It has been known for some time that heterozygosity for an LDL receptor gene defect in familial hypercholesterolemia results in LDL cholesterol levels of 200% of normal in 1/500 persons in the population. More recently, it has been realized that apo B mutations can profoundly affect LDL cholesterol concentrations. In heterozygous hypobetalipoproteinemia individuals have one defective apo B allele and have LDL cholesterol levels of only 30-50% of normal. These people are 1/1000-1/1500 in the general population. In familial defective apo B-100 affected individuals are heterozygous for a mutant apo B allele resulting in an amino acid 3500 change of Arg→Gln. Apo B derived from this allele is defective in LDL receptor binding and tends to accumulate in plasma. These persons have increased LDL cholesterol levels of 150% of normal and occur with a frequency of 1/1000 to 1/500 in the population. In very recent studies, we have shown that significant defects exist at the apo B locus in as many as 1/5 persons in the population. HDL particles are formed in plasma by a rather complex set of events. The building blocks appear to be the apo A-I and A-II, phospholipid and free cholesterol. HDL size and amount is also determined by processing proteins such as lipoprotein lipase, hepatic triglyceride lipase, lecithin cholesterol acyltransferase, and cholesteryl ester transfer protein. Rare inborn errors of metabolism have been described that affect HDL cholesterol concentrations, but do not explain the variation in HDL concentration in the population. Based on metabolic turnover studies of HDL apolipoproteins, we have found that on a fixed high fat diet HDL cholesterol concentration correlates best with apo A-I fractional catabolic rate and when changing from a low to a high fat diet the increase in HDL cholesterol levels correlates best with the change in the apo A-I synthetic rate. Using transgenic mice that oversynthesize the human apo A-I gene, we have shown a direct effect of this gene on HDL cholesterol concentrations and on dietary responsiveness of HDL cholesterol levels. Genes controlling apo A-I fractional catabolic rate on a high fat diet and those controlling apo A-I synthetic rate in response to dietary fat may harbor the loci to explain the common genetic variation in HDL cholesterol levels.

K 018 CELLULAR AND MOLECULAR APPROACHES TO HYPERTENSION AND CORONARY HEART DISEASE. A.G. Motulsky, R. Ward, P. Billings, S. Deeb, J. Brunzell, W. Burke, Departments of Medicine and Genetics, University of Washington, Seattle, WA 98195.

The public health impact of hypertension and of coronary heart disease is considerable. Both conditions show familial aggregation which is genetically determined. However, genetic studies are difficult because of gene-environment interaction and etiologic heterogeneity. Current approaches involve genetic study of major sources of variation of intervening phenotypes, i.e., investigations of possible major genes affecting pathophysiologic processes that predispose to these conditions.

In hypertension, we found red cell Na-Li countertransport (CT) to be consistently elevated in Caucasian but not in black hypertensive subjects. Extensive family studies showed that 80% of CT variation was genetic in origin and 20% environmental. One-half of the genetic variation could be ascribed to undefined polygenic factors and one-half to a recessive major gene with a population frequency of 25% which in the homozygous state (6-7% of the population) elevated CT to twice the normal level. The CT gene accounted for 15-20% of the attributable risk of systolic hypertension but contributed less than 5% to the variation of blood pressure in the normal range. The nature of the underlying gene mutation affecting CT remains unknown as does its chromosomal locus.

The molecular genetics of lipid characteristics has been studied in investigations of coronary atherosclerosis. We could find no association between RFLPs of apolipoproteins (A1-PstI, C3-SstI, A4-XbaI, A2-MspI, C2-TaqI, B-TaqI, B-Pvu2, B-EcoR1, and B-minisatellite) and hyperlipidemias (cholesterol, triglycerides, LDL cholesterol, apolipoproteins AI, AII, and B) except that a common variant of apolipoprotein B (XbaI) was associated with slight elevation of apo B and cholesterol levels. No association between any RFLP of apolipoproteins and coronary heart disease was detected. Current research focuses on apo B structure and regulation by genetic linkage and sequence studies.

Protein Structure

K 019 **THE CHEMICAL SYNTHESIS APPROACH TO PROTEIN MOLECULAR BIOLOGY**

Stephen B.H. Kent, Div. Biology, California Institute of Technology, Pasadena, CA 91125. Fundamental to the genetic origins of disease is an understanding of the way in which genetic lesions affect the occurrence or activities of the gene products, biologically active proteins. This presentation will give an overview of chemical peptide synthesis as the basis of a powerful new approach to the molecular biology of proteins. The methodology innovations that have led to this new approach are focused around the development of rapid, high efficiency automated chemical synthesis of peptides and proteins and, more recently, the construction of long peptide chains by ligation of large unprotected segments (domains). Systems in which the synthetic approach has been successfully applied in our laboratory include: the identification of immunodominant B- and T-cell epitopes, and the host cell receptor structure, in the hepatitis B virus envelope proteins; structure-function studies in interleukin-3, including the identification of an essential disulfide bond; the identification of a minimal "core" fragment with the biological activities of GM-CSF; and, the identification and synthetic replication of the AIDS virus binding site within the CD4 protein. The synthetic approach is also being used to study the HIV-1 protease, an enzyme essential to the replication of infective virions. Results of protein engineering of the HIV-1 protease by means of total chemical synthesis will be presented. This has led to an improved understanding of the molecular origins of the substrate specificity of this enzyme, and the systematic development of inhibitors.

K 020 THE NATURE OF PROTEIN DYNAMICS IN SOLUTION, Michael Levitt* and Ruth Sharon, Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel and *Department of Cell Biology, Stanford University, Stanford, CA, USA

Simulation of the molecular dynamics of a small protein, bovine pancreatic trypsin inhibitor (BPTI), is found to be more realistic when water molecules are included than when *in vacuo*: the time-averaged structure is much more like that observed in high resolution x-ray studies, the amplitudes of atomic vibration in solution are smaller, and far fewer incorrect hydrogen bonds are formed. We use this stable simulation to provide a description of the nature of protein dynamics in terms of the rates and amplitudes of atomic motion, time-varying patterns of hydrogen bonds and distinct conformational substates. Our approach, which provides a sound basis for reliable simulation of diverse properties of biological macromolecules in solution, uses atom-centered forces and classical mechanics.

K 021 PROTEIN CRYSTALLOGRAPHY, F. A. Quiocho, Howard Hughes Medical Institute, Departments of Biochemistry and Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030

The three-dimensional structures of biological macromolecules — assemblies (e.g., ribosome), proteins, enzymes and DNA — is the basis of our understanding all biological and biochemical processes at the atomic level. For these reasons, research in the area of structural biophysics has been and will continue to be of prime importance. X-ray crystallography provides the best means by which the spatial locations of atoms in these structures and the atomic interactions between proteins and substrates can be determined unambigously and completely. The pioneering studies in the 1950s of the three-dimensional structures of myoglobin, hemoglobin and DNA are landmarks in science, paving the way to the considerable advances in the field of crystallography and the enormous contributions to molecular biology. There are now approximately 400 protein structures determined. This field of research is enjoying its second stage of rapid growth, partly due to its primordial contribution to biotechnology. Protein engineering, molecular modelling and dynamics, site-directed mutagenesis, and drug and vaccine designs are some of the direct beneficiaries of crystallographic investigations of biological macromolecules. With three-dimensional structures on hand, drugs that bind to DNA and target proteins, enzymes and viruses are being rationally designed. All these benefits will continue to accrue. In my talk, I will briefly describe the method of x-ray crystallography and highlight recent applications of this method in a variety of biotechnological studies.

Diseases with Known Gene Products

K 022 MOLECULAR STUDIES OF ARGININOSUCCINATE SYNTHETASE AND CITRULLINEMIA, Arthur L. Beaudet, Peter J. Healy, Julie A. Dennis, Gail E. Herman, Marian J. Jackson, Linda C. Surh, Keiko Kobayashi, David B. Tick, Hope Northrup, and William E. O'Brien, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas USA.

Argininosuccinate synthetase (AS) is a urea cycle enzyme; genetic deficiency causes the disease citrullinemia. The human gene spans 65 kb, includes 15 exons, and maps to chromosome 9q34. The cDNA and amino acid sequence from the mouse, rat, cow, and human show extensive homology. There is extensive heterogeneity of mutations in human citrullinemia patients as determined by Southern blotting, Northern blotting, nuclease analysis of mRNA, and DNA sequencing. RFLPs at the argininosuccinate synthetase locus have been used for linkage analysis in the CEPH families and for prenatal diagnosis of citrullinemia. Citrullinemia has been identified in Friesian cattle in Australia. The bovine disease is due to an arginine (CGA) to nonsense (TGA) mutation in codon 86 and is associated with a profound reduction in the steady state level of mRNA. The bovine mutation results in the loss of an *Ava*II site which can be analyzed by PCR for screening of animals. The bovine model offers the opportunity for therapeutic trials using organ transplantation or gene therapy. Attempts to develop gene therapy for citrullinemia have resulted in the development of cell lines which produce recombinant retrovirus encoding the human cDNA sequence. Hepatocytes and bone marrow cells are feasible target tissues for gene therapy directed at treatment of citrullinemia. Bone marrow transplantation in mice using cells infected with the recombinant retrovirus results in expression of the human enzyme in mouse peripheral blood for 6-8 weeks following reimplantation.

K 023 SIMPLIFIED DIAGNOSIS OF NEW MUTATION X-LINKED DISEASES. C.T. Caskey, R.A. Gibbs, J.S. Chamberlain. Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.
The molecular diagnosis of several human X-linked diseases is complicated by a high frequency of new mutation events. We have developed techniques to rapidly screen for new mutations leading to the Lesch-Nyhan (LN) syndrome and Duchenne muscular dystrophy (DMD). These diseases represent contrasting challenges, as LN is often the result of a single DNA base change within the 651 bp peptide coding region of the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene, while DMD is frequently associated with large DNA deletions within the huge (>2 megabases) dystrophin gene. To facilitate point mutation detection in LN we have combined polymerase chain reaction (PCR) amplification of HPRT cDNA with automated direct DNA sequencing. Thus, a single DNA base difference in an expressed human DNA sequence can be identified by fully automatable procedures. To detect DMD deletions we have developed multiplex PCRs containing as many as seven individual sets of oligonucleotide primers that each amplify an individual DMD exon. It is therefore possible to can scan all deletion prone regions of the gene in a four hour procedure that requires no radioisotopes. These techniques have general application for other human genetic diseases.

K 024 LOCALIZATION OF A GENE FOR ATAXIA-TELANGIECTASIA TO CHROMOSOME 11q22-23, Richard A. Gatti, Ozden Sanal, Shan Wei, Patrick Charmley, Kenneth Lange, and Patrick Concannon*, UCLA School of Medicine, Los Angeles, CA 90024, and *Virginia Mason Research Center, Seattle, WA 98101. Ataxia-telangiectasia (AT) is a human autosomal recessive disorder of childhood characterized by: a) progressive cerebellar ataxia with degeneration of Purkinje cells; b) hypersensitivity to ionizing radiation; c) a greater than 60-fold increased cancer incidence; d) non-random chromosomal rearrangements in lymphocytes; e) thymic hypoplasia with cellular and humoral (IgA and IgG2) immunodeficiencies; f) elevated serum level of alphafetoprotein; and g) premature aging. A DNA repair enzyme is the suspected common denominator in this pathology. Heterozygotes are generally healthy; however, the sensitivity of their cultured cells to ionizing radiation is intermediate between normal individuals and that of affected homozygotes. Further, two recent reports document that heterozygous females are at an increased risk of breast cancer. These findings, when coupled with an estimated carrier frequency of 0.5 - 5.0 percent, suggest that a) as many as 1 in 5 women with breast cancer may carry the AT gene and that b) the increased radiation sensitivity of AT heterozygotes may be causing radiation therapists to reduce the doses of radiation used for treating cancer in all patients. In order to identify the genetic defect responsible for this multifaceted disorder, and to provide effective carrier detection, we performed a genetic linkage analysis of 31 families with AT affected members. This has allowed us to localize a gene for AT to chromosomal region 11q22-23 utilizing probes for thy-1 with a maximum LOD score (Zmax) of 4.34 at a distance (Θ) of 0.10 and PYNB3.12 (Zmax=5.58; Θ= 0.08).

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K 025 MOLECULAR GENETICS OF THE FACTOR VIII GENE AND HEMOPHILIA A. Jane Gitschier, Barbara Levinson, Scott Kogan and Susan J. Kenwrick. Howard Hughes Medical Institute, Department of Medicine, University of California, San Francisco, CA 94143.
Hemophilia A is an X-linked bleeding disorder caused by a defect in blood coagulation factor VIII. The 2351-amino acid protein is produced in small quantities by the liver and undergoes an extensive proteolysis by thrombin during its activation and inactivation. Hemophilia is clinically heterogeneous and therefore is likely to stem from a variety of mutations. We have examined the thrombin cleavage sites and associated acidic domains using two methods: discriminant oligonuculeotide hybridization, and denaturing gradient gel electrophoresis. A total of 5 mutations were found in over 200 samples and, in addition, a new polymorphism which will be useful for genetic diagnosis. Hemophilia often occurs in families without a prior history of the disease, reflecting the introduction of new mutations into the population. Isolated cases may also result from maternal mosaicism, thereby complicating genetic counseling. Three cases of such mosaicism for hemophilia have been discovered, one of which involves a partial deletion apparently arising from a duplication intermediate. In a separate line of research, a gene within the factor VIII gene has been characterized. This gene is contained entirely within intron 22 and is transcribed in the direction opposite to that of factor VIII. The abundant 1.8-kb mRNA is expressed in many cell types and its sequence is compatible with protein synthesis. A second transcribed copy of this gene is present on the X chromosome within 1.5 Mbp of the factor VIII gene. The function of either of these expressed sequences is as yet unknown.

K 026 GENETIC DEFECTS IN STEROID METABOLISM, Perrin C. White, M.D., Division of Pediatric Endocrinology, Cornell U. Medical College, New York, NY 10021.
Defects in the synthesis or metabolism of the steroid hormone cortisol may have adverse effects on sexual differentiation, growth, salt balance and ability to withstand stress. The inherited inability to synthesize cortisol is termed congenital adrenal hyperplasia (CAH). Steroid 21-hydroxylase (21-OHase) deficiency, which causes 90% of cases of CAH, occurs in a severe "classic" form in more than 1:10,000 births and in a mild "nonclassic" form in up to 3% of certain ethnic populations. It is characterized by symptoms of androgen excess and, in severe cases, by a concurrent inability to synthesize aldosterone, leading to renal salt wasting and shock. The 21-OHase gene, CYP21B, and a 98% identical pseudogene, CYP21A, are located in the HLA major histocompatibility complex on chromosome 6p, adjacent to and alternating with the C4B and C4A genes encoding the 4th component of serum complement. Each gene is 3.1 kb long and contains 10 exons. About 1/4 mutations in CYP21B are net deletions of C4B and CYP21B due to unequal crossing-over during meiosis, whereas most of the remainder are mutations normally present in the CYP21A pseudogene that have apparently been transferred to CYP21B by "gene conversion". These mutations include frameshifts, nonsense mutations and missense mutations causing amino acid substitutions. Most other cases of CAH are caused by 11-OHase deficiency, which is characterized by increased synthesis of androgens and by salt retention and high blood pressure rather than salt wasting. The 11-OHase (CYP11B) gene is located on chromosome 8q along with a 2nd homologous gene of unknown function. Each gene is 6.5 kb long and contains 9 exons. Although 21- and 11-OHase are both cytochrome P450 enzymes, their sequences are less than 20% identical. The 11-OHase enzyme also has 18-OHase and 18-oxidase activities (also called corticosterone methyloxidase I and II). Isolated 18-oxidase (CMO II) deficiency prevents synthesis of aldosterone, leading to salt wasting with no symptoms of androgen excess. This disorder is closely linked to polymorphisms in CYP11B. Thus, very different disorders apparently result from different mutations in a gene for a multifunctional enzyme. Another genetic defect in steroid metabolism involves corticosteroid 11-dehydrogenase, an enzyme that converts cortisol to cortisone. Defects in this enzyme allow cortisol, which is present at much higher levels than aldosterone, to occupy the renal mineralocorticoid receptor, leading to a rare, potentially fatal form of high blood pressure termed apparent mineralocorticoid excess. The amino acid sequence of 11-dehydrogenase predicted from rat and human cDNA is surprisingly similar to several other dehydrogenases in bacteria and invertebrates, thus identifying potentially important amino acid residues in this enzyme.

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DNA Repair

K 027 XERODERMA PIGMENTOSUM, A DEFICIENCY IN NUCLEOTIDE EXCISION REPAIR, James E. Cleaver, Louise H. Lutze, Audrey N. Player, Thai Nguyen, and William F. Morgan, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750.

Ultraviolet sensitivity of mammalian cells appears to be regulated by two major gene families that are recognized in different biological contexts. One family is associated with the human repair deficient disease xeroderma pigmentosum (XP groups A-I, V); the other with ultraviolet sensitive mutants of Chinese hamster ovary and other rodent cells (CHO groups I-VII). Human and hamster cells contain genes of both families, but the overlap between these families is unknown. Their functions can be analyzed through gene cloning, analysis of revertants, chromosome mediated gene transfer (CMGT), and the use of antisense oligonucleotides. Some genes are involved in regulating repair of subgenomic regions, especially those in XP group C and CHO group 1. Transformation of XP cells of most groups may increase the relative importance of an S phase repair process, which is associated with sensitization to caffeine. The XP variant (V) group may represent a deficiency in this S phase repair process or in excision repair of damage near the replication forks. Two independent XP group A revertants excise only the minor (6-4) UV photoproducts and are deficient in excision of cyclobutane dimers. The (6-4) products and not cyclobutane dimers therefore play a major role in the toxicity of UV damage in genomic DNA. Cyclobutane dimers however appear more important in damage to shuttle vector DNA. XP revertants can repair mammalian viral DNA (herpes virus) but not shuttle vector DNA which has been grown in E. coli. This suggests that some recognition factors involving DNA secondary structure in addition to the damaged sites are important in regulating repair in XP cells and that these factors are involved in XP reversion. Human genes which correct CHO complementation groups 1 and 2 have been cloned and show sequence similarities to RAD 2, RAD 10, and UVR genes from E. coli and yeast. Antisense oligonucleotides to the complementation group 1 gene confer UV sensitivity to wild type CHO cells and can be used as probes to analyze the role of this gene in hamster and human cells. Cloning of XP genes has not thus far been achieved because of a variety of technical and biological problems. Preliminary evidence for the XP gene size has been obtained through radiation inactivation during CMGT. These experiments suggest that chromosomal fragments approximately 200 kb in length from CHO cells are needed to correct the UV sensitivity of complementation groups A, C, and D of XP. The presence of CHO DNA in hybrid cells derived by CMGT was confirmed by the polymerase chain reaction, which demonstrated CHO alu-equivalent DNA sequences. Correction of the XP phenotype by hamster chromosome fragments represents an initial step in experiments which may lead to eventual isolation and cloning of members of the XP gene family.

This work was supported by the U.S. Department of Energy, contract no. DE-AC03-76-SF01012, and NIEHS Research Service Award 5T32-ES07106.

K 028 CHARACTERIZATION AND EXPRESSION OF EUKARYOTIC GENES REQUIRED FOR NUCLEOTIDE EXCISION REPAIR:YEAST AS A MODEL SYSTEM. Errol C. Friedberg, Lee Bardwell, A. Jane Cooper, Itzik Harosh, Wolfram Siede and Jae-Mahn Song, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

The yeast *Saccharomyces cerevisiae* has been extensively characterized with respect to the genetics of cellular responses to genomic injury. More than 30 distinct genetic loci have been identified which confer normal responsiveness to DNA damage. Among these at least 10 genes are involved in the removal of bulky base adducts that cause distortion of the DNA helix, a process referred to as nucleotide excision repair (NER). This genetic complexity suggests that in eukaryotes NER is effected by a multiprotein complex involving the products of many genes. Five of these genes (*RAD1, RAD2, RAD3, RAD4* and *RAD10*) are absolutely required for damage-specific recognition and damage-specific incision of DNA in vivo following exposure of cells to agents such as UV radiation. These 5 genes have been cloned by phenotypic complementation or, in one case (*RAD4*) by chromosome walking from a closely linked locus. The nucleotide sequences of these genes predict the expression of proteins with calculated molecular weights of 126.2 kDa (Rad1), 117.7 kDa (Rad2), 89.7 kDa (Rad3), 87.1 kDa (Rad4) and 24.3 kDa (Rad10). The cloned genes have been tailored into vectors for overexpression in yeast and *E. coli*. The *RAD3* gene is multifunctional. In addition to its role in nucleotide excision repair *RAD3* is an essential gene. Furthermore, certain *rad3* mutant alleles (characterized in other laboratories) confer a phenotype of increased spontaneous mutations and increased mitotic recombination, but do not result in significant hypersensitivty to UV radiation. Rad3 protein has been purified to apparent homogeneity. The purified protein is a DNA-dependent ATPase with DNA helicase activity. The role of this ATPase/helicase in the various *RAD3* functions identified remains to be established. To this end defined mutant alleles have been isolated and cloned into expression vectors for purification and characterization of mutant proteins. The *RAD2* gene is DNA damage-inducible. Following exposure to a variety of DNA-damaging agents steady-state levels of *RAD2* mRNA increase ~3-6 fold. Induction is positively regulated. Cis-acting sequences required for induction have been identified by deletion mapping and mutants have been isolated that are defective in induction of *RAD2*. The amino acid sequences of the *RAD10* and *RAD3* genes show significant similarity to the human excision repair genes *ERCC1* and *ERCC2* respectively. Additionally, the *RAD10* gene partially complements the phenotype of mammalian cells defective in the *ERCC1* gene. Hence, it would appear that genes for NER are conserved in eukaryotes and that yeast is a useful paradigm for investigating the biochemistry of this process.

K 029 DYNAMICS OF THE E. COLI UVR DNA REPAIR SYSTEM, L. Grossman, S.J. Mazur, P.R. Caron, E.Y. Oh and T.W. Seeley, Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205
The pre-incision steps governed by the E. coli nucleotide excision pathway include ATP-promoted UvrA dimerization followed by a somewhat indiscriminate process of nucleoprotein formation involving UvrAB complexes. This binding reaction is associated with localized topological unwinding of DNA. Localized unwinding precedes, and is required for, the 5' to 3' translocation of the repair complex. Translocation is sensitive to the presence of UV damaged sites. The energy required for this UvrAB dependent translocation appears to be generated by a UvrB-specific cryptic ATpase. UvrB protein can be converted to a 70 Kd truncated protein with ATPase activity, UvrB*, by a heat-shock induced protease that also acts on the E. coli Ada protein. UvrB* binds with UvrA to DNA but fails to participate to helicase or incision reactions.

K 030 **ROLE OF GENE EXPRESSION IN THE FINE STRUCTURE OF DNA DAMAGE PROCESSING,** **Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020**
DNA damage and repair are non-uniform in the mammalian genome, with preferential processing of some types of lesions in actively transcribed sequences. Such heterogeneity in repair may render invalid the use of overall genomic repair measurements to predict particular biological end points in mutagen-treated cells since the consequences of DNA damage depend upon its precise location with respect to relevant genes. For example, differences in repair efficiency for particular protooncogenes may affect the carcinogenic response in tissues in which those genes are differentially expressed. The selective repair of active, essential genes may be required to facilitate transcription and maintain viability. Replication would appear to be less critically affected since persisting lesions are found in daughter DNA duplexes and do not seem to greatly affect cell survival.

Cyclobutane pyrimidine dimers are removed much more efficiently from the transcribed DNA strands than from the non-transcribed strands in UV-irradiated mammalian cells, and Dr. Isabel Mellon has recently shown that even *Escherichia coli* exhibits this phenomenon, adding credence to the likelihood that it may be of general importance and that an excision repair complex may be coupled to the transcription apparatus. (Supported by NIH grant CA 44349.)

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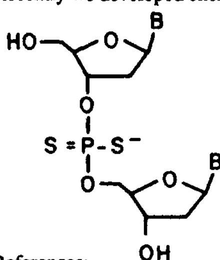
K 031 STUDYING MUTAGENESIS IN CELLS FROM INDIVIDUALS WITH GENETIC REPAIR DEFICIENCY DISEASES, M.M. Seidman, D. Brash*, S. Seetheram*, K. Kraemer*, A. Bredberg*. Otsuka Pharmaceutical Co., LTD., 9900 Medical Center Drive, Rockville, MD., 20850, *National Cancer Institute, NIH, Bethesda, MD, 20894.
A shuttle vector plasmid carrying a bacterial suppressor tRNA marker gene was treated with ultraviolet radiation and passaged through cultured skin cells from a patient with Xeroderma pigmentosum (XP) and also in repair proficient cells. Plasmid survival and mutations which inactivated the marker gene were scored by transforming an indicator strain of *E. coli*. Sequence analysis of mutant plasmids with single base changes revealed the prominence of the GC-AT transition in both cell types in agreement with the Adenine Insertion rule of Tessman. The XP cells (complementation group A) had a mutation hotspot not seen in the repair proficient line, while another, different hotspot was seen with cells from a group D XP cell line. The location of the major photo products in the tRNA gene was determined, and the frequency of modification was compared to the frequency of mutations at specific sites. No obvious correlation between mutation hotspots and modification hotspots was seen. We interpret this as a reflection of the influence of local DNA sequence on the balance between lethality and mutagenesis which results from ultraviolet modification of DNA. These results are reminiscent of those described by Fuchs for frameshift mutations induced by a hydrocarbon mutagen in *E. coli*, and this may be a general feature of mutagenesis induced by DNA damage. Recently, we have analyzed the mutational spectrum obtained after passage of the UV modified vector through additional cell lines. A striking feature of these experiments is the appearance of hotspots not seen in the previous studies. This occurs in repair deficient cells, suggesting this cannot be explained by differential repair. It seems likely that the appearance of mutagenic hotspots is a complex function of the DNA sequence, adduct-DNA structure, and cellular enzymology and physiology.

Drugs and Probes from Biotechnology

K 032 SYNTHESIS AND BIOCHEMICAL STUDIES OF DNA ANALOGS, Marvin H. Caruthers, Wolfgang K.-D. Brill, Yun-Xi Ma, William S. Marshall, John Nielsen, Henri Sasmor, and Jin-Yan Tang, Department of Chemistry and Biochemistry, University of Colorado, 80309
Recently we developed chemical methods that for the first time have allowed us to prepare DNA containing phosphorodithioate internucleotide linkages.[1-4] Because these synthetic procedures are completely compatible with the phosphoramidite methodology[5] for synthesizing DNA on silica supports, DNA segments can now be synthesized which have either any predetermined combination of phosphorodithioate and natural phosphate linkages or exclusively phosphorodithioate moieties. This analog has many properties that render it extremely useful for studying biochemical processes. It is isosteric and isopolar with the normal phosphate diester linkage, is easily derivatized with reporter groups, and, as part of synthetic DNA duplexes is recognized by repressors and *E. coli* DNA polymerase (Klenow fragment). Homopolymers containing this linkage have been shown to inhibit reverse transcriptases *in vitro* (AMV and HIV) and are resistant to degradation with snake venom and spleen phosphodiesterases.



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K 033 THE DEVELOPMENT OF RECOMBINANT HUMAN ERYTHROPOIETIN, Daniel Vapnek, Jeffrey Browne, Joan Egrie and Thomas Strickland, Amgen Inc., Thousand Oaks, CA 91320.
Erythropoietin (EPO) is the sialoglycoprotein hormone which regulates the level of red blood cell production. EPO is synthesized in the kidney and is transported, via the blood stream, to the bone marrow where it stimulates precursor cells to differentiate through the erythroid lineage into mature red blood cells. EPO isolated from urine contains N and O linked carbohydrate chains which terminate in sialic acid. Removal of the sialic acid causes a loss of *in vivo* biological activity. For this reason, a mammalian host, Chinese Hamster Ovary (CHO) cells was used for cloning and expression of the EPO gene. Analysis of recombinant human EPO produced in CHO cells has demonstrated that the recombinantly produced protein has a very similar carbohydrate structure to natural EPO. A variety of analytical methods have been used to compare EPO isolated from natural sources to EPO produced by CHO cells. By all methods used, the two molecules show a high degree of similarity.

Preclinical and clinical trials of recombinantly produced EPO have demonstrated its usefulness in a number of settings. These range from correcting the severe anemia associated with chronic renal disease, to its potential use in reducing blood transfusions in patients undergoing surgical procedures.

Genetic Engineering

K 034 ANALYSIS OF RETROVIRAL VECTOR EXPRESSION IN MURINE HEMATOPOIETIC CELLS, John W. Belmont, Kateri A. Moore, Maurizio Scarpa, Grant MacGregor, Fred Fletcher, and C. Thomas Caskey, Howard Hughes Medical Institute and Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030. Replication-defective retrovirus vectors which carry coding sequences for human adenosine deaminase (hADA) have been used to evaluate methods for gene transfer and expression in murine hematopoietic progenitors. The packaging cell line GP+E-86 allowed efficient production of these vectors without recombinant replication-competent virus as assessed by marker rescue and a novel PCR assay. Virus titers were between 10^5 and 10^6 IU/ml when determined by immunoperoxidase staining of target cells using a monospecific anti-hADA antibody. Murine bone marrow was infected with one of these vectors (which uses the MoMuLV LTR promoter) and then analyzed for expression of hADA *in vitro* and in reconstituted irradiated recipient animals. FACS analysis showed that up to 70% of the bone marrow cells expressed the hADA enzyme initially after infection. 75% of transplanted recipients had hADA in RBC's for at least 7 weeks. Western analysis of tissues from animals 3 weeks post-transplantation showed hADA in all hematolymphoid organs. Loss of expression of hADA was associated with absence of cells bearing the vector provirus as demonstrated by Southern and PCR analysis. The influence of hematopoietic growth factors on infection efficiency of CFU-S progenitors was investigated. Stimulation of bone marrow cells with conditioned media containing IL-3 and IL-6 gave optimal results. Expression of hADA in cell subpopulations resulting from infection with this vector and from vectors carrying lymphoid-specific enhancers is currently being analyzed by two-color FACS. These experiments focus attention on optimization of pluripotent hematopoietic stem cell infection in order to obtain efficient and stable expression of foreign genes in transplant recipients.

K 035 A Strategy for Generating Mice of Any Desired Genotype Through Gene Targeting. M.R. Capecchi, S. Mansour and K.R. Thomas, Department of Biology, University of Utah, Salt Lake City, Utah

Gene-targeting (homologous recombination of DNA sequences residing in the chromosome with newly introduced DNA sequences) in mouse embryo-derived stem (ES cells) promises to provide a means to generate mice of any desired genotype. First, standard recombinant DNA techniques are used to introduce a desired mutation into a cloned DNA sequence of a chosen locus. Then, that mutated sequence would be transferred into an ES genome by gene targeting. Microinjection of the mutant ES cells into mouse blastocysts could then be used to generate germ-line chimeras and finally, interbreeding of heterozygous siblings would yield animals homozygous for the desired mutation. A major technical obstacle to realization of this approach has been the unavailability of practical methods for obtaining ES cell lines carrying desired but non-selectable targeted mutation at loci of interest. We have developed a positive and negative selection procedure that enriches two thousand-fold for ES cells that contain a targeted mutation. The procedure was applied to the isolation of $hprt^-$ and $int\text{-}2^-$ mutants, but it should be applicable to any gene. A cloned fragment of the gene must be available and the intron-exon boundaries within that fragment defined. No other information is required.

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Oncogenes, Developmental Defects

K 100 A COMPREHENSIVE GENE ORDER FOR HUMAN CHROMOSOME 19Q, L. L. Bachinski and M. J. Siciliano. M.D.Anderson Hospital and Tumor Institute, Houston, TX. 77030.

Human chromosome 19 contains a number of genes of known medical importance, including the Low-Density Lipoprotein Receptor (LDLR), the Insulin Receptor (INSR), Apolipoproteins CI,CII and E (APOCI,APOCII,APOE), Hormone-sensitive lipase (HSL), chorionic gonadotropin-beta (CGB), and myotonic dystrophy (DM). In addition, there are a number of genes of potential importance in carcinogenesis such as transforming growth factor beta (TGFB), protein kinase C-gamma (PKCG), cytochrome p450 families A and B (CYP2A, CYP2B) and three DNA repair genes (ERCC1,ERCC2,XRCC1). Although the q arm of this small chromosome is marked by about 20 known genes and an additional 20 or more anonymous markers, no clear gene order has emerged in spite of extensive linkage studies. We present a gene order for the q arm of chromosome 19 based on a series of over 30 independent somatic cell hybrids in which the chromosome is broken, as shown by the presence of some, but not all, chromosome 19 markers. These hybrid lines, originally selected for the presence of various DNA repair genes, comprise a regional assignment panel for human chromosome 19 which has become a valuable resource for ordering the known markers on this chromosome. This work was supported in part by NIH grants CA09299 and CA34936 and gifts from the Exxon Corporation and Mr. Kenneth D. Muller.

K 101 THE CONTRIBUTION OF GENE OVER-EXPRESSION TO TRISOMY 18 PHENOTYPE. Mark A. Balacs, Charles H. Rodeck and Gudrun E. Moore, Queen Charlotte's Maternity Hospital, London W6 0XG, UK.

Trisomy 18 (Edwards') syndrome is the second most frequently recorded aneuploidy sufficiently compatible with intra-uterine life to allow peri- and post-natal survival (overall incidence of 1:7000). It is invariably associated with physical and mental retardation as well as with generally lethal anatomical defects. The project described here will test the hypothesis that the over-expression of genes located on chromosome 18 gives rise to the Edwards' phenotype. It is intended to complement the studies using quantitative dosage analysis on Northern blots with specific tissue localization using *in situ* hybridization. The blots will be probed and analyzed for over-expression, initially using myelin basic protein cDNA, a candidate gene mapping to 18q22-q23. To our knowledge, no investigation has been undertaken which utilizes the *in situ* hybridization of radioactively-labeled complementary RNA probes to mRNA in fixed tissue sections. The proposed study should reveal gene dosage effects at the transcriptional level, and by the careful age- and sex-matching of trisomic with normal tissue, the contribution of candidate gene over-expression to the observed phenotype will be estimated.

K 102 THE WAGR REGION: A PROBE, DELETION AND RESTRICTION MAP. Gail A.P. Bruns and Manfred Gessler, Genetics Division, Children's Hospital, Boston, MA 02115

The WAGR deletion region of chromosome 11p contains a constellation of genes implicated in development of the eye, brain and genitourinary tract as well as the Wilms tumor locus. To identify these genes and to assess the etiology of the overlapping deletions characteristic of this region, a bank of 40 DNA probes that map between proximal 11p14 and distal 11p12 have been developed by screening several phage libraries. More than 350 other chromosome 11 derived phage clones have also been identified. When regionally localized, these clones will provide a framework map of chromosome 11. Using gene dosage hybridization and 14 WAGR related deletions, the deletion region has been subdivided into 16 intervals. The aniridia and Wilms tumor subregions have been defined and a region of possible importance for the mental retardation component of the syndrome identified. The probes have been linked into 3 large clusters which span more than 14 megabasepairs of the WAGR deletion region by single and double digests with infrequently cutting restriction enzymes and PFG analysis. The breakpoints of an AGR deletion and a familial translocation associated with aniridia have been cloned and 5 additional WAGR related breakpoints identified by PFGE. In addition to a highly conserved region around the aniridia translocation breakpoint, 10 conserved sequence probes map at intervals between FSHB and the D11S9 locus. Four of these are located in the Wilms tumor subregion. Isolation of the corresponding cDNA clones should facilitate understanding of the role of these loci in the phenotype of the WAGR syndrome.

K 103 MODELS OF EARLY EMBRYOGENESIS - DIFFERENTIATING ES CELLS AND COLLAGEN GENE EXPRESSION. K.S.E. Cheah[1], E. Lau[1], P. Au[1], & R.H. Lovell-Badge[2]. [1]Biochemistry Dept., Hong Kong University, Sassoon Rd., Hong Kong. [2]National Institute for Medical Research, London NW7 1AA, U.K. Pluripotential stem cells derived from normal mouse embryos (ES cells) may be used as a model for early postimplantation embryonic development because of their ability to differentiate into many cell types in culture. The coordinate expression of different collagen genes is important for the development of vertebrate form and abnormalities in collagen gene expression can result in severe skeletal and connective tissue defects in the embryo. In this study mRNA has been isolated from differentiating ES cells over a 42d period & the pattern of expression of 6 collagen genes [α1(I), α 2(I), α 1(II), α1(III), α1(IV) & α2(IV)] analysed by RNase protection assays. The results are compared with the expression of these genes in mouse embryos at 6.5d, 8.5d, 13.5d and 16.5d development. Addition of ascorbic acid to the culture medium affects the extent of differentiation of ES cells and also increases the level of collagen expression. There studies are important for the interpretation of the phenotypic consequences of collagen mutations to embryonic development and in animal models of connective tissue disease. This work is supported by the Arthritis & Rheumatism Council UK, the Croucher Foundation (HK) and Strategic Research Grants HK University.

K 104 PHYSICAL MAPPING STUDIES OF HUMAN CHROMOSOME 3, Matthew Jonsen[1], Paul Erickson[1], Robert Gemmill[3], Carol Jones[2], Stuart Gold[1], Jonathan Baskin[1], Marijka Sage[1], and Harry A. Drabkin[1,2], (1) Division of Medical Oncology, University of Colorado Cancer Center (2) the Eleanor Roosevelt Inst. Denver, CO 80262, and (3) S. W. Biomedical Res. Inst. Scottsdale, AZ 85251.
Human chromosome 3 consists of 200 million bp of DNA and represents 7% of the human genome. Several specific chromosome 3 rearrangements in malignant and developmental disorders have been described. We have developed an extensive collection of molecular probes and somatic cell hydrids which are being used in the construction of a physical map. Approximately 350 molecular probes have been developed using both flow sorted libraries as well as larger insert EMBL lambda libraries constructed from fragments of #3. Over 150 of these probes have been mapped to various degrees. Our hybrid mapping panel includes most of the specific rearrangements identified to date, as well as a series of radiation reduction hybrids containing integrated fragments. Pulsed field maps of selected regions are under construction. Examples of this work will be presented.

K 105 RFLP ANALYSIS OF CHROMOSOME 22 IN 82 CASES OF MENINGIOMA. Jan P. Dumanski, Guy Rouleau, V.Peter Collins and Magnus Nordenskjöld,Ludwig Institute for Cancer Research, Stockholm Branch and Dept. of Clinical Genetics, Karolinska Hospital, 104 01 Stockholm, Sweden. Neurogenetics Laboratory, Massachussetts General Hospital, Boston, MA 02114, U.S.A.

The constitutional and the tumor tissue genotypes of 82 patients with primary meningiomas has been analysed using 11 polymorphic markers on chromosome 22q; D22S9, IGLV, IGLC, D22S10, D22S1, MB, c-sis, p450, W110D, W13E, W24F. Retention of the constitutional genotype was seen in 31 cases (38%), while another 41 cases (50%) showed loss of one constitutional allele at all informative loci consistent with monosomy 22. The remaining 10 cases (12%) showed loss of heterozygosity in the tumor DNA at one or more chromosome 22 loci, and retained heterozygosity at other loci. In all these cases constitutional genotypes were retained at centromeric loci, while telomeric loci showed loss of alleles, consistent with variable terminal deletions of one chromosome 22q in the tumor DNA. No evidence for an interstitial deletion has been found. The smallest deleted region of one chromosome 22 was the part distal to the MB locus. These results indicate that the meningioma locus is localized within 22q12.3-qter and are in agreement with our earlier finding. This study, encompassing 82 patients, underlines the necessity of developing new probes within the region of interest.

K 106 HETEROGENEOUS EXPRESSION OF erbB-2 mRNA IN HUMAN BREAST CANCER, C. Richter King, Sandara M. Swain, Laura Porter, Seth M. Steinberg, Marc E. Lippman and Edward P. Gelmann of Molecular Oncology, Inc., 19 Firstfield Road, Gaithersburg, MD 20878 and Lombardi Cancer Center, Georgetown University, Washington, D.C.

Amplification and mRNA expression of the erbB-2 gene was analyzed in 61 samples of primary human breast carcinoma. In the 57 samples where RNA could be isolated four different expression level groups were identified. Comparison of hybridization signal with that for Beta-actin revealed that erbB-2 mRNA could not be detected in 6/57 (11%), was detected at normal levels in 32/57 samples (56%), showed four- to eight-fold overexpression in 8/57 samples (24%), and showed 16- to 128-fold overexpression in 11/57 samples (19%). Examination of the DNA of the same set of samples revealed 6/61 samples (10%) with distinct gene amplification and 6/61 samples (10%) with possible gene amplification. The highest levels of erbB-2 overexpression were associated with gene amplification, Samples with four- to 16-fold overexpression of the erbB-2 mRNA occurred without evident gene abnormalities. There was no association of erbB-2 expression or gene amplification with clinical stage of breast carcinoma or axillary lumph node involvement. The clear amplification of the erbB-2 gene may be associated with a significantly shorter time to treatment failure.

K 107 ABERRATIONS OF CHROMOSOME 7 AND 14 IN NORMAL, PRE-MALIGNANT, AND MALIGNANT T CELLS, Ilan R. Kirsch, Marc-Henri Stern, and Stan Lipkowitz, NCI-Navy Medical Oncology Branch, National Institutes of Health, Naval Hospital, Bethesda, MD 20814

Inversions and translocations of chromosomes 7 and 14 are the most common abnormalities found in the peripheral blood of apparently normal individuals. Morphologically very similar abnormalities have been associated with distinctive, sporadic lymphoid malignancies. In the disease ataxia-telangiectasia (A-T), patients maintain 1-5% of their peripheral T cells with an inv(7) and can develop an inv(14) or a t(14;14) in almost 100% of their peripheral T cells as a clonal population while still not developing a frank leukemia. We have analyzed in molecular detail (using standard cloning methods and polymerase chain reaction) the nature of the chromosomal breaks in normal, A-T, and malignant T cells. We will present data regarding the role of certain gene regions (immunoglobulin gene superfamily hybrids as well as putative oncogenes) implicated in the chromosomal aberrations, the mechanism of formation of these aberrations, and the nature of the predisposition of patients with A-T to develop certain of these aberrations.

K 108 MOLECULAR DISSECTION OF A MICRODELETION SYNDROME (MILLER-DIEKER) REVEALS FREQUENT SUBMICROSCOPIC DELETIONS, CONSERVED SEQUENCES, AND AN HTF ISLAND. D.H. Ledbetter, S.A. Ledbetter, P. vanTuinen, Y. Nakamura, D. Barker, and W.B. Dobyns. Baylor College of Medicine, Houston, TX 77030; Howard Hughes Medical Institute and University of Utah, Salt Lake City, UT; Medical College of Wisconsin, Milwaukee, WI. Miller-Dieker syndrome (MDS), comprised of abnormal neuronal migration in the cerebral cortex and characteristic dysmorphic features, is caused by visible or submicroscopic deletions in sub-band 17p13.3. We are constructing a detailed map of this telomeric region using somatic cell genetic techniques combined with conventional and pulsed-field gel electrophoresis. A total of 10 DNA markers have been precisely localized and ordered within a span of <3 Mbp. Of 15 MDS patients, 10 have cytogenetically visible microdeletions which include 3-10 markers, while 5 with normal high-resolution cytogenetic studies show molecular deletions of 3-5 markers. Two highly polymorphic VNTR probes, YNZ22 and YNH37, are consistently co-deleted in all 15 patients and are therefore valuable for diagnosis and prenatal diagnosis of this disorder. Pulsed-field analysis of the smallest submicroscopic deletion with the flanking probe 506 showed altered fragment sizes with SfiI, NotI and NruI, allowing us to estimate the deletion size as >600 kb. The finding that all patients show large molecular deletions suggests at least two loci in the region must be deleted to produce the complex phenotype (i.e., it is a contiguous gene syndrome). Both YNZ22 and YNH37 cross-hybridize with rodent DNA indicating they are evolutionarily conserved sequences. An HTF island was identified surrounding YNZ22, an additional indication of the presence of an expressed sequence in this region. Both probes map to mouse chromosome 11, which contains a cluster of 5 independent neurological mutations mapping near other 17p markers. This raises the intriguing possibility that a cluster of genes involved in neurological development may reside in human 17p13.

K 109 HUMAN AND EQUINE HEREDITARY MULTIPLE EXOSTOSES: RFLP ANALYSIS USING CHROMOSOMAL AND ONCOGENE PROBES, Joseph K.-K Li, Brian Moloney, Eldon Gardner, James L. Shupe, Yoko Elsner, and Nicole C. Leone, Molecular Biology Program, Department of Biology and Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah 84322.
DNAs isolated from both normal and hereditary multiple exostoses-affected humans and horses were digested by 14 different restriction enzymes before the potential presence of restriction fragment length polymorphisms (RFLPs) was determined using seven oncogene and three chromosomal probes. Hybridization was detected in most human cases but no distinct polymorphisms could be found. However, distinct Bgl II RFLPs were detected in the DNAs of several male and female members of an HME family when c-sis oncogene and chromosomal probe pMS3-18 (located at q 11.2 - q 13 of chromosome 22) were used. pMS3-18 probe revealed a single two-allele polymorphism with fragments at either 6.5 or 4.7 kb. Frequency of distribution of these unique RFLPs were also considerably different between male and female members within this human kindred 203. With equine DNA samples, no hybridization was observed with V-ras oncogene probes. Similar hybridization patterns were detected with c-raf-1, c-fes, c-myb and c-sis oncogene probes. Unique RFLPs were only detected with c-raf-1 probe in Bam HI and Pst-1 digested equine DNA samples after extensive screening.

K 110 ISOLATION OF HUMAN GENES FROM RODENT X HUMAN SOMATIC CELL HYBRIDS, Pu Liu, and Michael J. Siciliano, Department of Molecular Genetics, University of Texas M.D.Anderson Cancer Center, Houston, TX 77030.
Primary transcripts of eukaryotic genes (sometimes referred to as hnRNAs) contain introns. One of the features of introns is that they have a greater chance of containing repetitive sequences than mature mRNA does. Since repetitive sequences are species specific, they could be used as a tag to isolate human genes from rodent x human somatic cell hybrids. A CHO x human somatic hybrid cell line 20XP3542-1-4 was used to test this possibility. This cell line was chosen because its human genome component has been well characterized. It has a fragment of chromosome 19 long arm as the only human genetic material. Several genes on the human chromosome fragment have been cloned including ERCC1, ERCC2 and XRCC1. Total cellular RNA was isolated from the cell culture and poly-A$^+$ RNA fractionated. Northern blot shows an enrichment of CHO repetitive sequences and human repetitive sequences were detected using radiolabeled total Hela DNA as probe. A cDNA library is being constructed. An oligonucleotide complementery to the 5' junction of the splice site was used as primer for the cDNA synthesis to ensure each cDNA made will contain an exon. The library will be screened with human total DNA probe and single copy parts of the positive cDNA clone will be analyzed by blot hybridizations and used to screen a general cDNA library to obtain cDNA of the mature mRNA copy. (Supported in part by a Rosalie B. Hite Fellowship, N.I.H. grant CA34936 and a gift from Mr. Kenneth D. Muller)

K 111 THE ATCC AND THE NIH REPOSITORY OF HUMAN DNA PROBES AND LIBRARIES- RESOURCES FOR GENETIC ANALYSIS - DONNA R. MAGLOTT AND WILLIAM C. NIERMAN, AMERICAN TYPE CULTURE COLLECTION, 12301 PARKLAWN DRIVE, ROCKVILLE, MD 20852.

In September, 1985, the NICHD and DRR established a repository for human DNA probes and libraries at the ATCC. Chromosome specific libraries developed by the Los Alamos and Livermore National Laboratories, primate libraries developed in Japan, and probes and cloned genes from international sources have been preserved, characterized, and made available. The ATCC also has extensive collections of clones and libraries generated from other genomes, hosts and vectors. Considering these resources together, the ATCC has available over 85 libraries, 75 oncogenes, 625 other probes and clones, 135 hosts, and 550 vectors. Many of the clone are closely linked to or can be used to detect deletions and amplifications at known disease loci.

K 112 CHARACTERIZATION OF A TRANSLOCATION INVOLVED IN VON RECKLINGHAUSEN NEUROFIBROMATOSIS. Anil G. Menon[a], Donna C. Rich[b], Michael A. Schmidt[c], Bernd R. Seizinger[a], Guy A. Rouleau[a], David H. Ledbetter[b] and James F. Gusella[a]. [a]Massachusetts General Hospital, Boston, MA 02114. [b]Baylor College of Medicine, Houston, TX 77030 [c]Mayo Clinic, Rochester, MN 55905.

The genetic defect causing von Recklinghausen neurofibromatosis (*NF1*) has been mapped to the proximal long arm of chromosome 17 by linkage analysis. Flanking markers have been identified, bracketing *NF1* in 17q11.2, and laying the foundation for isolating the disease gene. Recently, a family has been identified in which a mother and her two children show both symptoms of NF1 and presence of a balanced translocation t(1;17) (p34.3; q11.2). We have examined the possibility that the translocation has occurred in or near the *NF1* gene by constructing a somatic cell hybrid line containing only the (1qter-1p34.3::17q11.2-17qter) derivative chromosome. On chromosome 1, the breakpoint occurred between *SRC2* and *D1S57*. The translocation breakpoint was localized on chromosome 17 between *D17S33* and *D17S57*, markers which also flank *NF1*. These data are consistent with the possibility that the translocation event is the cause of *NF1* in this pedigree. Consequently, the isolation of the translocation breakpoint, by approaching from either the chromosome 1 or chromosome 17 side, may provide a short-cut to the identification of the *NF1* gene.

K 113 CONSTRUCTION OF A LIBRARY FROM HUMAN Xq24 -> qter INCLUDING THE Xq27 FRAGILE SITE IN YEAST ARTIFICIAL CHROMOSOMES, Max Muenke, Jim Shero, Philip Hieter, and Robert L. Nussbaum, Howard Hughes Medical Institute, Univ. of Pennsylvania, Philadelphia, PA, and Johns Hopkins Univ. Baltimore, MD.
In order to establish a long range restriction map around the fragile site at Xq27, we are in the process of constructing a library from the distal long arm of the human X chromosome in yeast artificial chromosomes (YAC). Source DNA is derived from a hamster x human somatic cell hybrid with Xq24 -> qter including the Xq27 fragile site as its only human material (Nussbaum et al, AJMG 23: 457, 1986). Yeast vectors differ from the ones previously described (Burke et al, Science 236:806, 1987) by containing yeast Y elements and an ochre suppressing form of a tRNA gene, SUP11, which allows the use of a color colony assay to assess the stability of the resulting YACs (Hieter et al, Cell 40:381 1985). Partial fill-in of BamHI digested hybrid DNA only allowed ligation to to partially filled-in SalI digested DNA. All reactions were done in agarose cell plugs to prevent shearing of high molecular weight DNA. Ligation products were size-selected on a preparative CHEF gel and directly transformed into yeast spheroplasts. Analysis from a pilot experiment of transformed yeast clones on CHEF gels revealed YACs with sizes mostly ranging from 100 - 300 kb. In addition to bona fide YACs with the appropriate vectors on either end, other classes of YACs were observed. Experiments are in progress to increase transformation efficiency, sizes of YACs and to eliminate other classes of YACs.

K 114 LINKAGE STUDIES ON FAMILIES SHOWING A RARE FORM OF SPINA BIFIDA
Robert Newton, Olafur Jensson*, Alfred Arnason*, Deborah Henderson and Gudrun Moore, Institute of Obstetrics & Gynaecology, RPMS, Queen Charlotte's Maternity Hospital, London, W6 OXG, U.K. *Genetics Division of the Blood Bank, National University Hospital, Reykjavik, Iceland.

Common neural tube defects such as spina bifida and anencephaly show a high prevalence in the general population (>1/1000 births). There is a large degree of variation in the incidence rates, with factors such as urbanisation, geographic area and genetic components all contributing to the overall aetiology. Because of this complex multifactorial aetiology we are confining our research effort to studing X-linked families in which the phenotype can be solely attributed to a genetic component.

We report here the progress on this ongoing linkage study. Three apparently X-linked pedigrees are being investigated. The largest (53 people) and therefore the most informative is an Icelandic family. Linkage data using this family has excluded a large part of the X chromosome, extending from approximately Xp22 to Xq24. At present the most positive LOD score is with probe dic56 (DXS143) localised to Xp22. It is intended to pursue this region using the two smaller families for new information with dic56 and other probes in the vicinity. Cosmid libraries are being constructed from total genomic DNA and probed with close markers including dic56 to produce more informative probes in this area. It is anticipated that these strategies will enable exciusion or linkage to this region of the X chromosome potentially involved in neural tube fusion.

K 115 POOR PROGNOSIS IN BURKITT'S LYMPHOMA (BL) FROM PAPUA-NEW-GUINEA (PNG) CORRELATES WITH SPECIFIC c-mye REARRANGEMENT. P. SMITH, S. Jones, T. Sculley, D. Moss. Queensland Institute of Medical Research and Department of Pathology, University of Queensland, Herston Road, Brisbane, Queensland, Australia 4006.
EBV-associated BL is endemic in both Africa and PNG. A recent epidemiological study in PNG revealed an extremely poor survival (less than 10%) for children with BL. This contrasts with an approximately 50% survival for African children with BL who are given similar treatment in a similar social and medical setting. While there may be several explanations for this, a study was undertaken to determine if there is a biological difference between PNG and African BL using 4 PNG and 7 African BL lines using molecular probes to the 8:14 translocation breakpoint region. Several differences have been documented, however the most consistent of these is a rearrangement seen in the 2nd exon of c-mye in 4/4 PNG and 1/7 African samples. Studies are continuing to determine the effect of this on c-mye transcription and on the c-mye gene product.

K 116 DETECTION OF A SPECIFIC RNA TRANSCRIPT ASSOCIATED WITH CHRONIC MYELOID LEUKEMIA USING A NOVEL ENZYMATIC PROCESS FOR THE AMPLIFICATION OF NUCLEIC ACIDS, Stephanie DeGrandis, Graham Henderson, Roy Sooknanan, Michael Twist, Kate Matthews*, Armand Keating* and Richard Smith, Cangene Corporation, Mississauga, ON, L4V 1T4 and *University of Toronto and Toronto General Hospital, Toronto, ON, M5T 2S8 CANADA
A nucleic acid sequence based amplification (NASBA-TM) process has been developed which exponentially amplifies specific nucleic acids in vitro. The homogenous reaction procedes at a single temperature and efficiently generates more than 100 million copies of each template molecule in 4 hours or less. The target is amplified specifically with perfect fidelity as determined by slot blot hybridization and sequencing respectively. We have applied this technology to the diagnosis of chronic myeloid leukemia (CML). The 8.5 kb chimeric mRNA formed by the fusion of the bcr and abl genes was readily detected using 1ng of total cellular RNA extracted from bone marrow cells or patient blood. Slot blot hybridization analysis and hydroxyapatite chromatography employing a ^{32}P-labeled probe specific for the bcr/abl splice junction confirmed the identity of the amplified product. This amplification procedure may be suited for monitoring the effectiveness of therapeutic agents used to treat bone marrow cells destined for autologous marrow transplantation procedures.

Gene Mapping and DNA Sequencing

K 200 USE OF PRIMER ELONGATION WITH RESTRICTION ENZYME DIGESTION TO INCREASE THE AMOUNT OF SEQUENCE GENERATED FROM A SINGLE TEMPLATE. D. Amorese, T. Norris, and M. Kirsch, E. I. du Pont de Nemours & Co. (Inc), Medical Products Department, Wilmington, DE 19898. A new sequencing strategy has been developed for the GENESIS™2000 that allows more sequence to be determined from a single stranded template. Initially the universal sequencing primer is used to obtain the first 350 to 400 bases of sequence. Then a second reaction is performed using the same template and primer, however this time only dNTPs are incorporated initially. This is used to synthesize complementary strands of approximately 350 nucleotides in length (nucleotide limiting) which are then labeled and terminated with the standard dNTP/ddNTP-F mixture. This produces fragments of approximately 350 to 750 nucleotides in length. These DNAs are digested with restriction endonucleases (that cleave near base 350 determined from earlier sequence data), and these fragments are analyzed on the GENESIS™2000. This process can be repeated multiple times to generate large amounts of sequence information from a single template without the need to synthesize new primers.

K 201 ANALYSIS OF DNA MELTING POLYMORPHISMS, A POWERFUL ALTERNATIVE TO RFLPs ANALYSIS. Olivier ATTREE, Dominique VIDAUD, Michel VIDAUD, Serge AMSELEM and Michel GOOSSENS, INSERM U.91, Laboratoire de génétique moléculaire, Hôpital Henri Mondor, 94010 Créteil, France.We recently described a simple and rapid procedure that allows the detection of DNA polymorphisms through the analysis of the melting behavior of PCR (Polymerase Chain Reaction) amplified genomic DNA fragments by Denaturing Gradient Gel Electrophoresis (DGGE). This procedure represents an attractive alternative to RFLPs analysis since i. the detection of the polymorphisms does not depend upon the alteration of a restriction enzyme site, ii. DNA melting polymorphisms constitute a multiallelic system and are therefore expected to be highly informative in linkage studies, iii. the technique allows the rapid characterization of point mutations, when coupled with direct sequencing, as we have shown in hemophilia B patients.

We demonstrate that it is possible to enhance the sensitivity of detection of this procedure by taking advantage of the presence of additionnal bands in the DGGE pattern of heterozygotes. We show, using allele specific oligonucleotide hybridization, that these additionnal bands represent DNA heteroduplexes. These heteroduplexes are spontaneously created during the PCR amplification step by the annealing of two allelic DNA strands. Heteroduplexes contain single base mismatches, leading to a clear loss of stability, and therefore allow the detection of nucleotide substitutions which do not alter the melting behavior of the corresponding homoduplex. Moreover we show that heteroduplexes can be generated in homozygotes by mixing with a control sample of known sequence, followed by denaturation and renaturation. This procedure permits direct characterization of point mutations since heteroduplexes are created except if the tested sample and the control have the same sequence. This procedure thus represents an alternative to the allele specific oligonucleotide hybridization technique.

These techniques have broad applications in human genetics, we present their utilization for genetic counselling in several hemophilia B families that were not accessible to RFLP analysis.

K 202 EXTENDING THE SCOPE OF PRENATAL DIAGNOSIS OF X-LINKED RETINITIS PIGMENTOSA (XLRP)

Bower DJ,[1], Lindsay S,[1,2], Bhattacharya S,[1,2], Jay M,[3], Sealey P,[1]; [1] MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, UK; [2] Molecular Genetics Unit, Newcastle University, 19 Claremont Place, Newcastle Upon Tyne, NE2 4AA, UK; [3] Moorfields Eye Hospital, London, UK.

XLRP is probably associated with two independent loci, RP2 and RP3, mapping to Xp2.1 and Xp11.3 respectively. A DNA marker, L1.28, shows close linkage with RP2. This probe has been uninformative with a number of individuals and families. We are using PCR to sequence L1.28 and the corresponding sequences in affected individuals, to detect mutations which are not revealed by RFLP mapping. These results should more precisely define the linkage between L1.28 and RP2 and possibly extend the scope of carrier detection and prenatal diagnosis for this condition.

K 203 DETECTION OF CHEMILUMINESCENCE FROM BIOLOGICAL MOLECULES USING SENSITIVE CCD (CHARGE COUPLED DEVICE) CAMERAS, Mike A.W.Brady, Richard P.Glover, John Morse, Martin F. Finlan, Malcolm J.Downes, Corporate Research, Amersham International plc, White Lion Road, Amersham, Bucks, HP7 9LL, England.
With the increasing use of chemiluminescence labelling technologies it is appropriate to investigate low light imaging detectors other than film. These may yield greater sensitivity, faster results, as well as quantification and automation etc. This poster describes the use of CCD camera technology in both cryogenic (liquid nitrogen) and intensified configurations. Each has advantages both with respect to each other and to film. Their characteristics and application to enhanced chemiluminescence and other light generating systems will be covered. In particular, the visualisation and quantification of single copy genes from human genomic DNA by Southern blotting will be demonstrated.

K 204 ANALYSIS OF EUKARYOTIC GENE EXPRESSION *IN SITU* USING A REPORTER GENE ACTIVATED BY CHROMOSOMALLY-LOCATED TRANSCRIPTIONAL AND TRANSLATIONAL SIGNALS, Daniel G. Brenner, Sue Lin-Chao and Stanley N. Cohen, Stanford University, Stanford, CA 94305. In order to identify and study the expression of cell cycle-regulated genes as well as those involved in cellular senescence, a series of vectors (SIN/*lac*) has been constructed to allow activation of a reporter gene carried by a retrovirus upon integration of the virus into an expressed region of the chromosome. The retroviral-based system is designed to fuse the *lac* gene to chromosomally-located promoters, thus serving as an *in vivo* probe for regulatory sequences. The choice of the *E. coli lacZ* gene as the reporter also allows the formation of enzymatically-active *in vivo* fusions of β-galactosidase (β-gal) with eukaryotic proteins, and hence the analysis of translational, as well as transcriptional, control. Self-inactivating (SIN) retroviruses that lack both the enhancer and the promoter of the LTR have been employed as vectors to allow maximal expression of the reporter gene. The expression of β-gal activity in cos-7 cells infected transiently by SIN/*lac* constructs carrying a known promoter adjacent to the disabled retroviral LTR has demonstrated the validity of the approach. Helper cell lines producing the otherwise cryptic SIN/*lac* viruses have been cloned by using the polymerase chain reaction (PCR) to detect the viral RNA in the supernatant of the clones. Live cells have been isolated using a fluorescence-activated cell sorter for the phenotypic expression of β-gal and then subsequently screened for the retroviral sequences by PCR analysis. Additionally, co-cultivation of SIN/*lac*-producing Psi-2 cells together with mouse NIH3T3 cells has resulted in the activation of β-gal expression in the target cells. Experiments currently are in progress to investigate the utility of this technique in the isolation of genes induced by serum starvation of mouse NIH3T3 cells.

K 205 CONSTRUCTION OF JUMPING AND LINKING LIBRARY AND THE LONG RANGE MAPPING OF HUMAN CHROMOSOME X, Jian-hua Chai, Institute of Genetics, Fudan University, Shanghai, People's republic of China, In order to establish the long range physical map of human chromosome X, we have made a jumping and linking library with rare cutter restriction endonuclease Not I. For this purpose, we constructed a new jumping and linking vector and target plasmid. The jumping vector was constructed with EMBL3AB Ava I fragment containing amber mutation AB and a right arm of NM1151 cut with Ava I partial digests. This vector, we call pJF-1, is a insertion vector to avoid the missing of small fragment from the target cell genome, its capacity is 0 12kb. The target plasmid was modified of pMLS12 with a Not I site replacing the BamH I site, the suppressor was containing in which. The linking cloning vector we used is EMBL3AB replacing the Bamh I site with Not I site, we call pLF-1. The genome DNA we prepared from human cell line containing 5 pieces of chromosome X. This cell were lysis in situ in agarose block. The DNA digested in the same condition with Not I to complete. The Not I fragment was ligated to the Not I digested plasmid pMLS 12 and cut with BamH I, this digests was ligated with BamH I cuted Jumping vector pJF-1. The jumping clone were selected by suppressor. The genome DNA was digested with Sau3A-I, the 20kb fragment in length of average were selected by gradient centrifugation and ligated to the plasmid pMLS12, cut with Not I and ligated with pLf-1. The linking clone were selected by suppressor.
The long range mapping will performed by chromosome jumping and pulsed field gradient gel electrophoresis.

K 206 **DIRECT ISOLATION OF EXPRESSED SEQUENCES FROM DEFINED CHROMOSOMAL REGIONS.** Laura Corbo, David L. Nelson, Maureen F.Victoria and C. Thomas Caskey, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030. We have developed a new approach to isolate human-specific expressed sequences from defined chromosomal region using somatic cell hybrids. In the example described here a hybrid cell line, X3000-11 retaining the human Xq24-qter in rodent background, has been used as source of human transcripts. This technique involves the use of nuclear RNA to synthesize cDNA primed by oligonucleotides derived from human *Alu* sequences. Assuming that a large fraction of human unspliced nuclear RNA will carry repeated sequences of the *Alu* family, it is possible to direct the synthesis of human cDNAs using specific human primers. Many different oligonucleotides derived from human *Alu* sequences have been tested and one oligo, TC65, was found to be able to prime the synthesis of human cDNAs specifically. Because of the small amount of each cDNA the polymerase chain reaction (PCR) technique has been used to amplify the products. The experimental plan comprised: 1) Nuclear RNA extraction from X3000-11 and RJK-88 cell lines (RJK-88 is an hamster cell line used as negative control) 2) Synthesis of cDNA primed by *Alu*-oligo(s) 3) cDNA tailing with dGTP using terminal transferase 4) PCR with *Alu* and poly-C primers. PCR experiments confirmed the specificity of this approach; in fact there is DNA amplification in the X3000 nuclear cDNA, and no amplification in hamster nuclear cDNA used as control. Sequences amplified in this manner have been used to screen a λ library from flow-sorted X chromosome. Several positive clones have been isolated. The molecular analysis of these clones is in progress.

K 207 A RAPID CHEMICAL CLEAVAGE METHOD FOR THE DETECTION OF BASE CHANGES IN RNA. H.-H.M. Dahl, S. Lamande, R.G.H. Cotton and J. Bateman, The Murdoch Institute and Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia, 3052.

We have developed a new method for the detection and localization of mutations and polymorphisms in RNA. The method is based on the increased reactivity of mismatched cytidines and thymidines in heteroduplex DNA strands on treatment with hydroxylamine and osmium tetroxide, respectively. Treatment with piperidine then causes cleavage at the modified bases (1). After chemical treatment the DNA is electrophoresed on denaturing acrylamide gels and the resulting fragments identified by autoradiography. The fragment sizes localize the sites and types of the base changes. To extend this technique to the detection of base changes in RNA we formed a heteroduplex between the RNA (or cDNA) and a ^{32}P-end-labeled DNA fragment. Mutations in low abundance mRNA species were detected by amplification of specific regions of the cDNA by use of the polymerase chain reaction. This technique is being applied to a number of metabolic and connective tissue disorders. For example, a number of mutations affecting collagen gene expression, causing lethal osteogenesis imperfecta or Ehlers-Danlos syndrome, have been located and characterised. These mutations include point mutations, insertions and deletions. The chemical cleavage method has several advantages over previously published methods. So far all expected mutations have been detected and localized, and it is fast and technically simple.

1. Cotton et al, PNAS 85, 4397 (1988).

K 208 A SIMPLE PROCEDURE FOR DNA SEQUENCING BASED ON RANDOM IS1-PROMOTED DELETIONS, Istvan Fodor and Igor L. Glukhov, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, 142292, USSR.
Expression of oLpIN region of λ phage DNA inserted into the plasmid vector leads to inhibition of plasmid replication. For selection of spontaneous mutations in the cloned oLpIN region we have used a method based on this observation. Some 10% of all mutants have contained insertion of IS1-element. Using E.coli strain that complements insertional mutations in oLpIN region a method for a positive selection of IS1-promoted deletions in oLpIN region and its flanking sequences has been elaborated. We have demonstrated that this method can be applied for in vivo selection of a set of overlapping deletions in the cloned DNA fragments adjacent to IS1 segment. Deletions arising in oLpIN::IS1-containing plasmids exhibit the unique feature of extending from a fixed site located at the right terminus of IS1. This procedure offers significant advantages over the conventional ones in sequencing of long DNA fragments since it does not require laborious physical mapping and additional cloning of subfragments. Analysing the structure of the large number of random deletions we have observed specificity in location of the distal site of some deletions. Possible mechanisms of this specificity will be discussed.

K 209 FLUORESCENT DNA SEQUENCING USING DYE LABELED NUCLEOTIDE TERMINATORS, Steven Fung, B. John Bergot, Vergine Chakerian, J. Scott Eadie, Davis Hershey, Linda G. Lee, Steven M. Menchen, and Sam L. Woo, Applied Biosystems, Inc., 850 Lincoln Centre Drive, Foster City, CA 94404
The automation of DNA sequencing technology has undergone significant developments in the past few years. In particular, fluorescent based systems have had a major impact in advancing the technology. We have established a program to develop fluorescently labeled nucleotide terminators for the Applied Biosystems DNA sequencing system. Chain terminating nucleotides, each labeled with a unique fluorophore are incorporated using Sanger sequencing methods with different enzymes including T7 and Taq polymerases. Numerous dye nucleotide terminators have been prepared and examined to develop structure-activity relationships. A progress report illustrating our results in dye terminator DNA sequencing is presented.

Biotechnology and Human Genetic Predisposition to Disease

K 210 INHIBITION OF IN VITRO PRE-mRNA SPLICING BY ANTISENSE DEOXYOLIGONUCLEOTIDE ANALOGUES. Paul J. Furdon and Ryszard Kole, Department of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, N.C. 27514.
An in vitro transcribed human ß-globin pre-mRNA was hybridized to different antisense deoxyoligonucleotide analogues before treatment in a nuclear extract to block splicing in vitro. The deoxyoligonucleotide analogues contained either normal phosphodiester, alternating phosphorothioate, or alternating methyphosphonate inter-nucleotide linkages. Since intact pre-mRNA is required in a splicing assay, the hybrids were first tested for their susceptibility to RNase H from a HeLa cell nuclear extract or RNase H from E. coli. RNase H from both sources cleaved RNA in hybrids formed with deoxyoligonucleotides containing normal and phosphorothioate linkages but did not cleave RNA complexed with methylphosphonate derivatives.
Methylphosphonate deoxyoligonucleotide complementary to the 5' splice site hybridized to pre-mRNA at the expected position as shown by a primer extension assay and competition with a normal deoxyoligonucleotide with the same sequence in an RNase H assay. In an in vitro splicing reaction, the 5' splice site antisense methylphosphonate oligonucleotide inhibited splicing only if it was prehybridized to the pre-mRNA. This oligo-nucleotide when added after the start of the reaction or an oligonucleotide complementary to a sequence in exon 2 were unable to inhibit splicing. Experiments are in progress to see if a methylphosphonate oligo-nucleotide antisense to the 5' splice site inhibits splicing complex formation. In addition, a methylphosphonate oligonucleotide complementary to a pre-mRNA splicing mutant from a patient with ß+-thalassemia is being used to inhibit aberrant splicing. This pre-mRNA contains a mutant splice site which is used preferentially over the normal one. If aberrant splicing could be specifically inhibited, normal splicing should be restored.

K 211 A BIOSENSOR BASED ON SURFACE PLASMON RESONANCE : PRINCIPLES, PERFORMANCE AND APPLICATIONS. S A Charles, J C Irlam, M F Finlan, T Endericks, S E Garnham, A G Evans, D Pollard-Knight, P J Heaney, J C Corrie*, & P B Garland, Pollards Wood Laboratory, Amersham International plc, Little Chalfont, AMERSHAM, Bucks, England, HP8 4SP and *Medical Research Council Collaborative Centre, Burtonhole Lane, LONDON, England, NW7 1AD. Surface plasmon resonance (SPR) phenomenon can be utilised to detect the changes of refractive index that occur at a silver surface when one partner of a molecular binding pair diffuses from solution to bind to the other partner previously immobilised at that surface.[1] Examples of molecular binding pairs include antigen/antibody: lectin/glyco-protein: DNA/DNA: DNA/RNA: enzyme/substrate: DNA/DNA binding protein: hormone receptor/hormone and generally all ligand/ligand receptor interactions. The roles of the solution partner and surface partner are often interchangeable. If the solution partner is a molecule at the exterior of a cell, bacterium, virus or subcellular particle, then the range of applications can be further extended. Practical implications of SPR principles have required considerable inventiveness. Our current sensitivities are about 10^{-13} mole in the simplest unenhanced format. The obvious advantages of the basic method are lack of labels (fluorophores, radiochemicals, enzymes), fast response and simplicity of protocols. Considerable enhancements of sensitivity are achievable with the inclusion of refractive index probes. [1]Nylander, C., Leidberg, B., and Lund, T. (1982). Sens. Actuators, 3, 79-88. (Work supported by the DTI, and in collaboration with the Medical Research Council and Thorn-EMI).

K 212 MAGNETIC SOLID PHASE DNA SEQUENCING, E. Hornes[1], L. Korsnes[1] and M. Uhlen[2], [1]Research Division, Apothekernes Laboratorium A.S, Oslo; [2]Kungliga Tekniska Hogskolen, Biokjemiska Institusjonen, Stockholm

A new method for sequencing double stranded DNA using magnetic beads; Dynabeads™ has been developed.
This method is simple and rapid and lend itself to automation.
The general consept consists of binding DNA to magnetic beads. This can be done by end labelling fragments with Biotin and reacting with Dynabeads coated with Streptavidin.
The bound double stranded DNA is made single stranded by simply denaturation and magnetic separating.
Beads containing single stranded template is sequenced using small modifications of established dideoxy sequences procedures.

K 213 PHYSICAL MAPPING AND ISOLATION OF EXPRESSED SEQUENCES IN THE VICINITY OF THE HUMAN FACTOR VIII GENE, Susan J. Kenwrick, Barbara B. Levinson, Pamela H. Silvera, Mark N. Patterson* and Jane Gitschier, Howard Hughes Medical Institute, University of California San Fransisco, CA 94143 and Department of Oncology, Stanford University, Stanford, CA 94305*.

Several X-linked disease loci map genetically to the interval Xq27 to Xqter for which candidate genes have not been identified. One approach to finding expressed sequences is to identify CpG islands in the region of interest. We have isolated three CpG islands associated with the factor VIII (F8) gene at Xq28. One of these islands resides within a F8 intron and reveals a 1.8 kb mRNA transcript in cell lines from a variety of tissues (Levinson et. al. Am. J. Hum. Genet. 1988, 43 (3), pA192). A second CpG island, 700.1, situated 3.5 kb 5' to the promotor region of the F8 gene identifies two large, low-abundance transcripts with a specific distribution in the same cell lines. Two other unique sequences within 15 kb of 700.1 are associated with mRNA sequences that differ in size and cell line distribution from each other as well as from the transcripts detected by the island. The relationship of these sequences is being examined using cDNA clones isolated from appropriate libraries. A third CpG island, situated 30 kb from the 3' end of the F8 gene, does not detect a transcript in any cell lines so far examined. Isolation of sequence 700.1 has enabled us to refine the large scale physical map around F8 using pulsed-field gel electrophoresis. We have determined the direction of transcription of the F8 gene relative to that of the physically linked gene G6PD, and located two more CpG islands, 60 kb and 190 kb from 700.1.

K 214 A NOVEL HIGHLY SENSITIVE DNA LABELING AND DETECTION SYSTEM BASED ON DIGOXIGENIN: ANTI-DIGOXIGENIN ENZYME-LINKED IMMUNO-SORBENT ASSAY (ELISA) [1], Christoph Kessler, Hans-Joachim Höltke, Rudolf Seibl, Josef Burg and Klaus Mühlegger, Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg (FRG).

Besides the application of isotopes a number of methods have been developed for non-radioactive labeling and detection of nucleic acids. Most of the non-radioactive systems involve enzymatic, chemical or photochemical derivatization of DNA with biotin, and detection of the biotin-labeled DNA with (strept-)avidin coupled to marker enzymes. Although the sensitivity of DNA detection after enzymatic biotin-incorporation is rather high (< 1 pg) a considerable background may arise.
We have developed a novel highly sensitive non-radioactive system for specific detection of 0.1 pg DNA within 16 hrs in dot-, slot- or Southern-blots avoiding any significant background on nitrocellulose and nylon membranes even after colour development for 72 hrs. The high specificity of the novel system has also been used for the detection of single-copy genes in mammalian genomic DNA, colony and plaque hybridization as well as _in situ_ hybridization.
Especially in the latter application it is of great advantage, that the novel system avoids any significant background or side reactions with biological materials. In contrast to the wide-spread vitamin biotin, derivatization of DNA is achieved with the artificial steroid-hapten digoxigenin, a chemically derived aglycon of digoxin.

[1] Kessler, C., Höltke, H.-J., Seibl, R., Burg, J. and Mühlegger, K. (1989) Nucleic Acids Res., in preparation.

K 215 DIRECT SEQUENCING OF THE FACTOR IX GENE IN 21 FAMILIES WITH HEMOPHILIA B IMPLICATES CpG AS A DRAMATIC HOTSPOT OF MUTATION, Dwight D. Koeberl, Cynthia D. K. Bottema, Jean-Marie Buerstedde, and Steve S. Sommer, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905. Genomic amplification with transcript sequencing (GAWTS) is a three-step procedure that allows direct genomic sequencing.[1] Eight regions of the factor IX gene, which include the putative promoter region, the coding region, and the splice junctions, were sequenced using GAWTS. The sequence of all eight regions was obtained from 20 unrelated normal individuals of defined ethnicity and subsequently from 21 hemophiliacs in different families. The rate of polymorphism in these eight regions of functional significance is about 1/3 the average rate observed for intronic and intergenic sequences on the X chromosome, so the causative mutation should be the only sequence change seen in the overwhelming majority of hemophiliacs. In the observed mutations, the rate of C to T transitions at CpG is elevated by an estimated 62-fold. Nineteen point mutations (thirteen distinct mutations) were defined, as well as two small deletions. High quality reproducible sequence data can be obtained on a time scale that makes direct carrier testing and prenatal diagnosis feasible, and avoids multiple problems associated with current RFLP analysis.

1. Stoflet, E.S., Koeberl, D.D., Sarkar, G., and Sommer, S.S. _Science_ _239_:491-494 (1988).

K 216 A SIMPLE STRATEGY FOR SEQUENCING DOUBLE STRANDED DNA. S.Koepf[1], R. A. Gibbs[2], P, Nguyen[2], W. Salser[3], P. E. Mayrand[1], M. Hunkapiller[1], M. N. Kronick[1], L. J. McBride[1]. [1]Applied Biosystems, Inc., 850 Lincoln Centre Drive, Foster City, CA 94404, [2]Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, [3]Department of Biology, UCLA, Los Angeles, CA 90024. The fluorescence-based Sanger method of automated DNA sequence analysis has become a popular technology. Both single- and double-stranded template preparation and sequencing protocols exist. However, it is generally accepted that double strand sequencing can yield variable results. A promising solution is to convert double strand templates into a single stranded form with the use of the polymerase chain reaction (Gyllensten and Erlich, 1988, Proc. Natl. Acad. Sci. USA, 85:7652-6).

We have developed a simple sequencing procedure which employs direct Sanger sequencing of single-stranded polymerase chain reaction (PCR) products. Plasmid DNA, viral DNA, cDNA, and phage plaques all have been sequenced by a "universal protocol". We have sequenced PCR products directly without intermediate purification by the use of labeled sequencing primers. Furthermore, we have eliminated the need to synthesize template-specific sequencing primers by incorporating a universal primer sequence on the 5' end of the limiting PCR primer.

This procedure should ultimately become both a general template preparation method as well as a sequencing strategy for many high throughput and/or repetitive DNA sequencing projects.

K 217 AUTOMATED GENE DETECTION, Ulf Landegren, Robert Kaiser, Jason Stewart, Sara Mackeller, Steven Beall, and Lee Hood, Division of Biology, Caltech, Pasadena, 911 25.
DNA sequence detection assays can be expected to be performed at a greatly increased rate in both clinical and research contexts in the near future. We have recently developed a rapid, standardized assay with the capability to distinguish known sequence variants (Science 241, 1077 (1988)). In this technique, two oligonucleotides, complementary to contiguous segments of a DNA sequence, are used. If properly basepaired, the oligonucleotides may be joined covalently by a DNA ligase in a target-dependent fashion. The oligonucleotide ligation assay lends itself to automation. We are presently developing a prototype of an instrument, capable of analysing a hundred samples in a few hours. The positive identification of given DNA sequences result in an immediately detectable fluorescent signal. Strategies are being investigated to simultaneously analyse multiple genetic loci in the DNA samples. The technique may be applied to rapidly distinguish allelic forms of genetic markers for linkage analysis or for the unique identification of individuals.

K 218 IN SITU LINKAGE ANALYSIS BY SIMULTANEOUS HYBRIDIZATION OF MULTIPLE COSMID CLONES TO HUMAN METAPHASE CHROMOSOMES, Lichter, P. Tang, C.C. and Ward, D.C., Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510 A Suppression hybridization protocol (Lichter et al., Human Genetics 80, 224, 1988) has been used to rapidly map the chromosomal loci of human DNA fragments, cloned in cosmid or phage vectors, on metaphase chromosomes. In this procedure, genomic DNA clones are labeled with biotin, 2,4-dinitrophenol or other non-isotopic reporter molecules and then preannealed with an excess of unlabeled human competitor DNA prior to sample application. This preannealing step markedly suppresses the hybridization of repetitive DNA elements in the probe (such as SINES and LINES) and permits the visualization of the unique sequence components within the clone using direct fluorescent detection methods. Cosmids from human chromosomes 11 and 21, respectively, have been used to simultaneously localize multiple DNA fragments on a single chromosome by using differentially labeled probes in combination with two or more fluorochromes for detection. Selected sets of cosmids that are broadly distributed over the chromosome of interest are used together to establish an *in situ* linkage map with delineated anchor points. Inclusion of an unmapped cosmid clone from the same chromosome, labeled with a different reporter, into the "linkage-map-cosmid-set" permits the rapid localization of this DNA fragment within the linkage map. Initial studies indicate that resolution of this *in situ* linkage analysis is such that clones separated by less than 1-2 mega basepairs can be spatial distinguished.

K 219 A HIGHLY POLYMORPHIC (TG)n MICROSATELLITE AT THE D11S35 LOCUS. Michael Litt, Vikram Sharma and Jeffrey A. Luty. Departments of Biochemistry and Medical Genetics, Oregon Health Sciences University, Portland OR 97201. The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n=approximately 10 to 60. We and others (M. Litt and J.A. Luty. Am. J.Hum.Genet., in press; J.L.Weber and P.E.May, Am.J.Hum Genet 43:A161) have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. Using unique flanking primers and resolving the products on sequencing gels, these "microsatellite" VNTR loci may be detected by PCR. Since the distribution of "classical" highly polymorphic VNTRs tends to be biased towards subtelomeric regions, we have screened several clones previously mapped to interstitial regions to attempt ascertainment of highly polymorphic loci. Here we report that D11S35, previously mapped to 11q22, contains such a locus, with at least 6 alleles and a heterozygosity of 90%. Together with Weber and May's description of microsatellite VNTRs at the APOA2 (PIC=0.65; 1q21-q23) and APOC2 (PIC=0.79; 19q12-q13.2) loci, these results suggest that highly polymorphic (TG)n microsatellites may be readily ascertained in interstitial genomic regions. Because their full information content is accessible without haplotyping, such loci will be especially useful in mapping late onset disorders via affected sib pair and affected relative pair analyses.

K 220 **THE USE OF MOBILITY-SHIFTING NUCLEOTIDE ANALOGS TO DETECT DNA MUTATIONS AND POLYMORPHISMS**, Kenneth J. Livak and J. Stephen Kornher, Central Research & Development Dept., E. I. du Pont de Nemours & Co., Wilmington, DE 19880
We have devised a new, general procedure that will detect single nucleotide polymorphisms at any site in a DNA fragment, not just those affecting restriction sites. The method exploits the fact that the incorporation of certain nucleotide analogs into DNA causes a detectable shift in electrophoretic mobility. The method involves using a DNA polymerase to synthesize DNA strands of defined length, replacing one of the four dNTPs with a mobility-shifting analog. DNAs that are the same length but differ in the number of analog molecules per strand will exhibit different mobilities on a sequencing gel. This provides a rapid assay for distinguishing DNAs that may be identical in length but differ in base composition. To demonstrate this technique we have analyzed a nonsense mutation found in the gene encoding the human insulin receptor [Kadowaki *et al.*, *Science* **240**:787-790 (1988)]. Using PCR, a 140-bp segment containing the mutational site was amplified in genomic DNA isolated from a patient heterozygous for this mutation and from an homozygous normal individual. Each of the amplified DNAs was analyzed by primer extension using biotin-11-dUTP in place of TTP. On a sequencing gel, the primer extension product from the mutant allele migrates at a position one nucleotide slower than the product from the normal allele because the mutant product contains one more biotin-11-deoxyuridine residue than the normal product. This distinguishes the heterozygote from the homozygote. Similar analyses have been performed with an analog of dCTP.

K 221 HphI RFLP AND MAP OF POLYMORPHIC SITES AT THE RENIN GENE LOCUS, Masharani U, Nakashima P F, Cal Bio Inc., Mountain View, CA 94043.

9 RFLPs have been reported at the renin gene locus detected with restriction endonucleases BglI, BglII, HindIII, RsaI, TaqI, EcoT14I (two RFLPs), MboI and HinfI. We report another RFLP at this locus: HphI (GGTGA) detects a single dimorphism with presence or absence of a 0.7kb fragment (invariant bands at 1.2, 1.1, 0.9, 0.6, 0.5 and 0.4kb). The 0.7kb allele has a frequency of 0.23±0.04 when screened in 52 North American Caucasians. These 10 RFLPs have been mapped and they span a 20kb region around the human renin gene (figure 1). The RFLPs can be used to construct haplotypes and will be of value as markers for chromosome 1.

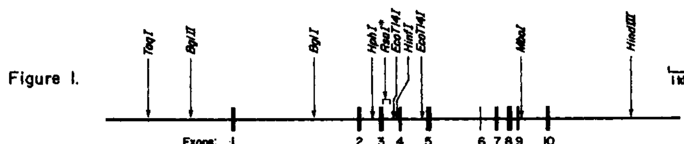


Figure I.

* RsaI RFLP exact location not known but lies in 150bp region indicated by horizontal bracket in figure 1.

K 222 RAPID KINETICS OF NUCLEIC ACID HYBRIDIZATION IN A THIN-LAYER CHROMATOGRAPHIC FORMAT, Michael E. McMahon and Julian Gordon, Diagnostics Division, Abbott Laboratories, North Chicago, IL 60064.
An ascending chromatographic system has been developed which allows for the rapid, specific hybridization of complementary DNAs under appropriate experimental conditions. In this format, a strip of nitrocellulose membrane, serving as the chromatographic medium, contains a capture bar of immobilized, denatured DNA and a dot of complementary oligonucleotide(s) positioned directly below the capture bar. Thin-layer chromatography is initiated by placing one end of the strip into an appropriate hybridization/running buffer. The oligomers, observed to migrate with the solvent front, interact with the single-stranded DNA at the capture site during the chromatographic process. Evaluation of hybridization signals generated at the capture bar reveals that specific hybridization occurs in less than three minutes in an excess of oligomer to capture DNA. Unreacted oligomers migrate with the solvent front to the top of the strip at the completion of chromatography. This inherent wash step therefore obviates the requirement for an independent wash of the membrane following hybridization. Overcoating of nitrocellulose strips to minimize nonspecific binding of mobile oligomers is not an absolute requirement. High specificity of hybridization is observed under relatively reduced stringency conditions. One may envision the application of this hybridization delivery technology to the diagnosis of infectious diseases, viral infections, human genetic disorders as well as the detection of other genetic markers of interest.

K 223 **ALU PCR: AMPLIFICATION OF HUMAN DNA SPECIFICALLY FROM HUMAN:RODENT HYBRID CELLS WITH APPLICATION TO THE HUMAN GENOME INITIATIVE.** David L. Nelson, Maureen F. Victoria, Laura Corbo, and C.Thomas Caskey, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030. Human:rodent hybrid cell lines are often utilized for isolation of human clones specifically from the region retained in the hybrid cell. This has required the production of recombinant libraries from the hybrid of interest and isolation of clones using human-specific repeat sequence probes. We have developed oligonucleotide primers that recognize human *Alu* repeats specifically, and have utilized these for PCR amplification of human sequences between *Alu* repeats. No amplification is observed from hamster or mouse DNA. The technique allows the isolation of a large number of sequences from the human region in the hybrid. In an example hybrid, containing the human Xq24-qter region as its only human material, a portion of the HPRT gene has been amplified, demonstrating amplification of a specifc human sequence from an expressed region. Amplification products can be used as probes for mapping and for isolation of cosmid or YAC clones from a total human library. Furthermore, the technique can provide a fingerprint type analysis of hybrid cell lines with small amounts of human sequence (1-50 Mbp) and of YAC and cosmid clones. The technique has broad applications for a variety of projects involving somatic cell genetics.

K 224 A HYBRIDIZATION TECHNIQUE UTILIZING PROBE DNA, DIRECTLY LABELLED WITH HORSERADISH PEROXIDASE, WHICH IS DETECTED BY ENHANCED CHEMILUMINESCENCE. Timothy C. Richardson and Michael A. W. Brady, Pollards Wood Laboratory, Amersham International plc, Amersham, Buckinghamshire, HP7 9LL, England. There are a number of technologies in use for the detection of labelled nucleic acid probes that have been hybridized to target nucleic acid on membranes. To date, probes have mainly been labelled with radioactivity, commonly ^{32}P incorporated into a nucleotide (eg dCTP). Latterly, other techniques have been introduced; these include the use of biotinylated nucleotides, hapten-labelled nucleotides or chemically modified nucleotides. An alternative approach is the direct labelling of nucleic acid probes using a complex of horseradish peroxidase[1]. The labelling procedure takes less than 20 minutes, following which the probes are hybridized to membranes at 42°C in an adapted hybridization buffer. Hybridized sequences are detected, after stringency washing, using a substrate system that generates a signal by enhanced chemiluminescence (ECL). This provides "hard copies" of the result on film, with exposure times of less than 1 hour. This system enables the detection of single copy genes in human genomic DNA; sensitivity levels of 1pg are achievable on Southern blots. The system has also been demonstrated to work on Northern blots, in RFLP analysis, for plaque screening and for colony screening. It is a method that will be of value to laboratories that need simple, rapid and safe hybridization technology.

(1) RENZ, M. and KURZ, C., Nucleic Acids Research, 12 (8), pp.3435-3444, 1984.

K 225 RAPID AND DIRECT ACCESS TO AN mRNA SEQUENCE OR ITS PROTEIN PRODUCT IS NOT LIMITED BY EITHER TISSUE OR SPECIES SPECIFICITY, Steve S. Sommer and Gobinda Sarkar, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905. RNA amplification with transcript sequencing (RAWTS) is a rapid and sensitive method of direct sequencing that involves cDNA synthesis, polymerase chain reaction (PCR) with a primer(s) containing a phage promoter, transcription from the phage promoter, and reverse transcriptase mediated sequencing (1). Here we show that each of four tissue specific human mRNAs examined can be sequenced by RAWTS from RNA isolated from each of the four cell types examined: white blood cell, liver, K562 erythroleukemia cells, and chorionic villus cells. These results indicate that there is a basal rate of transcription, splicing, and polyadenylation of tissue specific mRNAs in adult and embryonic cells. In addition to accessing sequence information, it is possible to generate a desired in vitro translation product by incorporating a translation initiation signal into the appropriate PCR primer. RAWTS can be used to obtain novel mRNA sequence from other species as illustrated with a segment of the catalytic domain of factor IX. Comparison of the sequences indicates that this segment of factor IX evolved at a rate equal to the average of a recent compendium of mammalian proteins. In general, the ability to obtain mRNA sequences rapidly across species boundaries should aid both the study of protein evolution and the identification of sequences crucial for protein structure and function.
(1) Sarkar, G. and Sommer, S.S. Nucleic Acids Res. 16:5197, 1988.

K 226 MAPPING OF THE Bcg MOUSE HOST RESISTANCE LOCUS. E.Schurr, E.Skamene, A.Forget, and P.Gros, Department of Biochemistry, McGill University, Montreal General Hospital Research Institute, Universite de Montreal, Montreal, PQ, H3G IY6, Canada.
Natural resistance of inbred mice to infection with Mycobacterium bovis(BCG) and Mycobacterium lepraemurium is controlled by the Bcg host resistance locus. Since the protein encoded by this locus is as yet undefined, we aimed at the identification of genetic markers closely linked to Bcg which can be used as a starting point for the cloning of the gene. Therefore, we have conducted a RFLP analysis in I86 backcross animals segregating at the Bcg locus as well as in 36 AXB/BXA, 26 BXD, and I3 BXH recombinant inbred strains (RIS). All backcross mice and RIS were typed for resistance or susceptibility to infection with Mycobacterium bovis (BCG). Genomic DNA was prepared from the livers of individual mice, digested with restriction endonucleases and analysed by Southern blotting to identify informative RFLPs. The probes used in our study were specific for Len2, Fn, Vil, Alpi, and Achrg, five genes which we had found to cosegregate with Bcg in the N20 generation of a Bcg congenic BIO.A(Bcg^r) strain. Detailed segregation and pedigree analysis indicated the gene order and inter-gene distances as: centromere-Len2-3cM-Fn-5cM-Vil,Bcg-6cM-Alpi-IcM-Achrg. No crossover was observed between Vil and Bcg in over 400 meiosis investigated. This observation places Vil extremely close to Bcg, possibly less than 200kb, and makes Vil the closest marker of Bcg reported to date. In addition, we noted that the genes which bracket Bcg on mouse chromosome I are precisely conserved on the telomeric end of human chromosome 2q. The identification of this syntenic group designates chromosome 2q as the primary target area for the search of a human homologue of the mouse Bcg host resistance locus. (Supported by MRC/WHO/DAAD).

K 227 SENSITIVE, CHEMILUMINESCENT NUCLEIC ACID DETECTION USING ALKALINE PHOSPHATASE TRIGGERED 1,2 DIOXETANE *A. Paul Schaap, **Denise Pollard-Knight, **Adrian C. Simmonds, **Mike A.W. Brady, and *H. Akhavan-Tafpi. *Department of Chemistry, Wayne State University, Detroit, MI 48202, **Corporate Research, Amersham International, White Lion Road, Amersham, Bucks HP7 9LL, England.

For the detection of complementary nucleic acids by specific hybridisation the poly nucleotide probe is most commonly radioactively labelled with ^{32}P. In the last few years, there has been an increasing demand for non-radioactive alternatives. We have therefore been investigating the use of chemiluminescent systems for nucleic acid detection. One of these involves the triggering of a stable 1,2 dioxetane by alkaline phosphatase. Enzymatic removal of a phosphate protecting group from the stable dioxetane produces an unstable intermediate which decomposes to generate light. The light can be detected using a luminometer, exposure to X-ray film or on a cooled charge collection device (CCD). The high sensitivity of this chemiluminescent system will be demonstrated by data showing detection of single copy genes in Southern blots of human genomic DNA. Typically exposures to X-ray film of between two and twelve hours are required to achieve this sensitivity. A unique feature of this chemiluminescent system is the stability of the lightoutput over at least seven days which allow repeated exposures over this period.

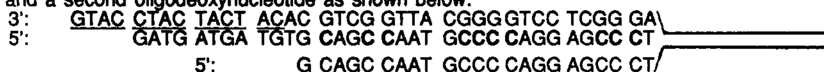
K 228 QUANTIFICATION OF POLYMERASE CHAIN REACTION PRODUCTS BY AFFINITY-BASED HYBRID COLLECTION, Ann-Christine Syvänen, Marina Bengtström, Jukka Tenhunen, Hans Söderlund, Orion Genetic Engineering Laboratory, Orion Corp. Ltd., Valimotie 7, 00380 Helsinki, Finland.
Identification of nucleic acid sequences by hybridization is a powerful tool for diagnosis. However, the sensitivity and specificity of present hybridization methods is often insufficient and the methods are cumbersome and yield only semiquantitative results. To solve these problems we devised a method in which the polymerase chain reaction (Mullis & Faloona, 1987, Meth. Enzymol. 155, 335) and the affinity-based hybrid collection procedure (Syvänen, et al. 1986, Nucleic Acids Res. 14, 5037) are combined. In this method oligonucleotides modified with biotin in their 5′-end are used as primers in the polymerase chain reaction. This results in the synthesis of 5′-biotinylated DNA molecules. These are detected by liquid hybridization to a labelled probe followed by collection of the formed hybrids on an avidin-matrix. The collected hybrids are measured and quantified with the aid of a hybridization standard curve prepared with varying amounts of biotinylated target DNA. Thus a quantitative measure of the DNA produced by the polymerase chain reaction is obtained. The procedure allows convenient identification of a very small number of DNA molecules of interest and using oligonucleotide probes mutations in the amplified DNA can be observed.

K 229 IMPROVEMENTS IN AUTOMATED DNA SEQUENCING WITH FLUORESCENT TERMINATORS, G. Tice, A. Hochberg, M. Gold, K. Wong, T. Norris, M. Kirsch, and D. Amorese. E. I. du Pont de Nemours & Co., (Inc.), Medical Products Department, Wilmington, DE 19898. The improvements to the GENESIS™2000 System can be separated into 3 categories: 1) Software--both the operating and base calling software have had several features added to enhance performance. Automatic lane finding, script electrophoresis (pre-programmed wattage changes at specified times), and automatic launch of base calling after the completion of the run, means that the operator need only start the instrument, apply the samples, and collect the data. The base caller has been improved to call more peaks with higher accuracy; to identify peaks that are not "computer called" accurately; and to simplify the visual editing process. 2) Molecular Biology--refinements in the protocols have lead to reactions that require only 5 minute incubations (single stranded templates) and 7 minute incubations (double stranded templates) after annealing. The option to use ethanol precipitation in place of the spin column for sample clean-up and concentration has also been developed for convenience. 3) Gel Resolution/Data Acquisition--improved alogrithyms in the software position the laser in the center of the lane (where the resolution is best) and reduced cross-linker gels, coupled with script electrophoresis result in higher quality data at a faster rate (450 nt in approximately 6 hours).

K 230 3'- AND 5'-FLUORESCENCE-LABELING OF DNA FOR SEQUENCING AND MAPPING, George L. Trainor, Frank W. Hobbs, and Mark A. Jensen, Paul R. Johnson, Ken J. Livak, and Peter Korolkoff, Central Research and Development Department and Medical Products Department, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, DE 19880. We have developed a stable of reagents for the fluorescence-labeling of DNA that are generally useful in the nucleic acid research and diagnostic areas. [*Science*, **238**:336-341 (1987)] Succinylfluorescein-labeled dideoxynucleotide triphosphates are substrates for a number of DNA polymerases as well as for terminal deoxynucleotidyl transferase and can be employed for 3'-dye-labeling of DNA in any context. Research applications include more powerful physical mapping techniques such as fluorescent fingerprinting. The succinylfluorescein dyes have also been incorporated into phosphoramidites that allow, for the first time, direct preparation of 5'-fluorescence-labeled oligonucleotides on automated DNA synthesizers. Such labeled oligonucleotides are useful as primers in the Cetus polymerase chain reaction. Applications include a comprehensive new method for polymorphism mapping.

K 231 RAPID GENETIC TESTING: ALLELE-SPECIFIC PRIMING USING THE POLYMERASE CHAIN REACTION, Anthony S. Weiss and Amanda Capes, Department of Biochemistry, University of Sydney, NSW, 2006, Australia Using polymerase chain reaction (PCR), present methods often rely upon additional steps (e.g. DNA probing or restriction enzyme cleavage) to detect subtle alterations in base sequence, such as those found in human inherited disorders. We describe here a novel method, called allele-specific priming (ASP), for the rapid and simple detection of base changes, which does not rely upon the additional steps described. ASP was made possible in theory by allowing differentiation between PCR primers which are perfectly matched, and those which have a single base mismatch. This was found to depend upon systematic and subtle adjustment of reaction conditions. The technique was also made more effective by adding a third primer to the system. Application of the method to a model system was successful, and work is proceeding with a common human point-mutation disorder. Results show great potential in the analysis of a wide range of genetic diseases, including the application of ASP to the rapid detection of restriction fragment length polymorphisms (RFLPs).

K 232 SINGLE STRANDED DNA DISPLACEMENT REACTIONS: CLONING OF A SPECIFIC DNA FRAGMENT HOMOLOGOUS TO BROMODEOXYCYTIDINE-SUBSTITUTED DNA, James G. Wetmur and Robin S. Quartin, Department of Microbiology, Mount Sinai School of Medicine, New York NY 10029. Substitution of a bromodeoxycytidine (BrdC) for deoxycytidine (dC) increases DNA-DNA hybrid stability by 0.17, 0.4 or 0.7 kcal/mol at pH 10, 7 and 4, respectively. BrdC-containing oligodeoxynucleotides (12-mer) will displace dC-containing strands from blunt-end duplexes with second-order rate constants of 1, 3 and 9 $M^{-1}sec^{-1}$ at 27, 32 and 37°C, respectively. If 4 complementary nucleotides are added to both the BrdC-containing strand and the complementary strand in the duplex, the rate constant increases to 440 $M^{-1}sec^{-1}$ at 27°C and becomes less dependent on temperature. This rate of displacement is of the same order of magnitude as DNA reassociation with nucleation limited to a 4-base region. A BrdC-containing oligodeoxynucleotide will transiently invade the ends of double-stranded DNA at a restriction endonuclease cleavage site containing an overhang. This product may be trapped by use of DNA ligase and a second oligodeoxynucleotide as shown below:



Underline: Kinased linker with SphI overhang. **Boldface**: BrdC.

The results may be summarized:

	Number of BrdCs	Relative Yield
PstI site	1	1
PstI site plus 4 nucleotides	3	>10
PstI site plus 19 nucleotides	10	>300

The ligated product contains an SphI site for the subsequent cloning step.

K 233 AUTOMATED ANALYSIS OF T-CELL RECEPTOR VARIABLE GENE EXPRESSION, Richard K. Wilson, Dwight Kono, Chia Chen, Annette Yuen, James Lindelien and Leroy Hood, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125

The expression of T-cell antigen receptor variable (V), diversity (D) and joining (J) gene segments has been found to be restricted in some types of T cell-mediated autoimmune diseases, and also may occur in other deleterious immune responses. In the future, it may be possible to selectively delete the responsible T cell subset using toxins which have been immunologically targeted at specific structures in the variable regions of the antigen receptors on the surface of these cells. As a prelude to such therapies, we have developed a procedure by which a large number of T-cell receptor structures from both normal and disease-associated systems may be rapidly analyzed. This system uses direct amplification of T-cell receptor-specific RNA from tissue and blood samples or T-cell clones, followed by subcloning and DNA sequence analysis. To facilitate the processing of samples, we have automated many of the molecular procedures, including the DNA sequencing reactions, using a modified robotic workstation. Using this analysis system we have investigated the restricted utilization of T-cell receptor variable gene components in mice afflicted with experimental allergic encephalomyelitis, determined the relative expression of V gene segment subfamily members in human thymic tissue and peripheral lymphocytes, and identified novel V and J gene segments in both mouse and human systems.

K 234 HUMAN CHROMOSOME-SPECIFIC PARTIAL DIGEST LIBRARIES IN LAMBDA AND COSMID VECTORS, Kathy Yokobata, Jennifer McNinch, Lee Pederson, Marvin Van Dilla and Pieter J. De Jong, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550.
We have developed procedures to extract, concentrate and clone DNA from specific human chromosomal fractions sorted by the Livermore high-speed sorter. The procedures have been employed successfully for the preparation of human chromosome-specific lambda and cosmid libraries for the following chromosomes: 19, 21, 22 and Y. The vectors lambda Charon 40 and lambda GEM 11 were used, while a new cosmid vector with two cos sequences (Lawrist 5) was constructed for the cosmid libraries. Lawrist 5 is derived from the lambda origin cosmid vector Lorist X, prepared by P. F. E. Little, containing the T7 and SP6 promoters immediately flanking the Bam HI and Hind III cloning sites. We have determined the library size (independent recombinants and approximate chromosome equivalents) for each lambda and cosmid library:

Chromosome	Library Size	Chrom. Equivalents	Chromosome	Library Size	Chrom. Equivalents
19 (Charon 40)	1.5×10^5	38	19 (Cosmid)	4.6×10^4	31
19 (Charon 40)	7.0×10^4	19	21 (Cosmid)	3.0×10^4	27
21 (GEM 11)	5.8×10^5	192	22 (Cosmid)	5.3×10^4	43
22 (GEM 11)	5.4×10^5	163	Y (Cosmid)	4.9×10^4	37
Y (GEM 11)	3.1×10^5	86			

Other characterization includes determining the library purity, average insert size, and some examination of the sequences present in the library. Additional libraries are presently being constructed for chromsomes 7 and 11. Work performed under auspices of the US Department of Energy, Contract No. W-7405-ENG-48.

Heart, Neurological and Metabolic Disease; Protein Structure

K 300 MOLECULAR AND CLINICAL CORRELATIONS OF DELETIONS LEADING TO DUCHENNE AND BECKER MUSCULAR DYSTROPHY, Lisa L. Baumbach, Jeffrey S. Chamberlain, Patricia A. Ward and C. Thomas Caskey, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030
Duchenne (DMD) and Becker (BMD) muscular dystrophy are allelic X-linked disorders that display great heterogenity in their clinical progression. These diseases are associated with a high frequency of new mutations which result in intragenic deletions in a majority of patients. To examine the relationship between genetic abnormalities and varying clinical phenotypes, we examined 160 affected males with human DMD cDNA probes. Deletions were detected in 56% of these patients, and were concentrated in two high frequency deletion regions (HFDRS) in the proximal and central portions of the locus. We have recently isolated genomic clones containing exons for the central HFDR which has allowed for the identification of exon:intron boundaries for eight consecutive exons. This information has permitted us to predict the effect that a deletion would have upon the translational reading frame of the resultant dystrophin mRNA. A comparison of molecular and clinical data for 26 DMD and BMD patients with deletions in this region indicated that apparently identical deletions could be associated with different disease phenotypes and that the predicted effects which deletions produce upon dystrophin translational reading frames do not always correlate with the associated disease. These results suggest that multiple mechanisms may be involved in producing these different disease phenotypes within the DMD locus. Further molecular analysis of these deletions is underway, as well as a determination of the specific sequences surrounding deletion breakpoints in the central HFDR in DMD and BMD patients.

K 301 GENETIC POLYMORPHISM OF RABBIT AND HUMAN N-ACETYLTRANSFERASE: MOLECULAR MECHANISM Martin Blum, Denis M. Grant, Anne Demierre and Urs A. Meyer, Department of Pharmacology, Biocenter, University of Basel, CH-4056 Basel, Switzerland
Arylamine N-Acetyltransferase (NAT) is the target of one of the most common (50 % of Caucasians) genetic polymorphisms. Phenotypically slow acetylators have been shown to be homozygous for an autosomal recessive gene. The NAT polymorphism confers marked interindividual variation in the disposition of numerous drugs (isoniazid, sulfamethazine, procainamide, caffeine etc.) but also potential carcinogens (benzidine, 2-aminofluorene, β-naphtylamine). A predisposition to a variety of human diseases including cancer has been associated with the NAT phenotype.
Due to the very low concentration of NAT in human liver we first purified the enzyme from rabbit liver. Rabbits provide an animal model for this polymorphism. Using an antibody raised against the purified rabbit protein we isolated and functionally expressed a full length cDNA clone from a λgt11 library, which was constructed from liver mRNA of an in vivo phenotyped homozygous rapid acetylator rabbit. In Western and Northern type experiments we could show that both protein and mRNA were below the detection level in liver of a homozygous slow acetylator rabbit as compared to intermediate and high levels in heterozygous and homozygous fast acetylator rabbit. Data from Southern blot analysis are consistent with a deletion mechanism causing the NAT defect. Internal amino acid sequence information from the purified human NAT is presently used to clone the human NAT gene. Initial experiments with the rabbit probes (which crossreact at the level of protein, RNA and DNA) indicate a similar mechanism for the human NAT-defect.

K 302 ISOLATION OF VIRAL AND VIRUS INDUCED HOST mRNAs BY DIFFERENTIAL HYBRIDIZATION, Jean-François Bureau, Sylvie Chirinian, Christine Aubert, Simona Ozden, Michel Brahic, Department of Virology, Pasteur Institute, Paris France 75724.
Theiler's virus isresponsible for a persistent infection of the central nervous system (CNS) of SJL/J mouse, accompanied by inflammation and primary demyelination. Virus replication in the CNS is minimal. The mechanisms of persistence and demyelination are only partially understood.
In principle, subtractive hybridization should allow the isolation of viral RNAs, and virus induced host mRNAs, in diseases of possible viral etiology. This approach was tested with the Theiler's virus model.
A cDNA library (lambda gt 10) was constructed with poly A+ RNA extracted from infected mouse CNS. The frequency of viral clones in the library was 1/15000. The library was screened with subtracted cDNA probes. 46 clones were obtained and analyzed. One of them contained part of Theiler's virus genome. Two cellular mRNAs, which were 10x overexpressed in infected tissues, were analyzed including by sequencing.
This study shows that subtractive hybridization should be powerful tool to study diseases of possible viral etiology.

K 303 DERIVED AMINO ACID SEQUENCE OF HUMAN SYNEXIN: A PROTEIN WITH CALCIUM CHANNEL ACTIVITY AND SIMILARITIES WITH LIPOCORTIN-LIKE PROTEINS, A.L. Burns, K. Magendzo, A. Shirvan, M. Srivastava, E. Rojas, M. Alijani and H.B. Pollard, Laboratory of Cell Biology and Genetics, NIDDK, NIH, Bethesda, MD 20892
Synexin is a calcium dependent membrane binding protein, which was initially identified by its ability to aggregate purified adrenal chromaffin granules. This protein later was shown to insert into lipid bilayers and to act as a voltage dependent calcium channel. We present data on the cloning and sequencing of synexin. Secondly, we show that the sequences of different synexin cDNAs reveal heterogeneity, which appears the result of using alternative splice and poly(A) sites. Thirdly, the predicted amino acid sequence from the synexin cDNAs is very similar to lipocortin-like proteins (calelectrin, calpactin, endonexin II, lipocortin I and porcine protein II). The N-terminal region of the lipocortin-like proteins are different and tend to be short, except for synexin which is longer (163 amino acids versus 16-46) and more hydrophobic. However, the synexin sequence in the C-terminal region contains four conserved repeats of about 70 residues each and has $45 \pm 5\%$ identity (except repeat 7 of calelectrin) with the other proteins. Each of these proteins bind to acidic phospholipids in a calcium dependent manner and this function is likely to reside in this domain(s).

K 304 MUTATIONS IN THE TRANSTHYRETIN (THYROXINE BINDING PREALBUMIN) GENE WHICH LEAD TO HUMAN DEPOSITION DISEASE. J.N. Buxbaum, D.R. Jacobson and P. Gorevic, N.Y. V.A. Medical Center, NYU Medical Center and SUNY at Stony Brook. Fibrillar tissue deposits in familial amyloidotic polyneuropathy consist of mutant forms of serum transthyretin (TTR) with substitutions at positions 30 (val→met), 33 (phe→ileu), 60 (thr→ala), 77 (ser→tyr) and 84 (ileu→ser). 4 of 5 have been confirmed by the detection of the predicted restriction digest change. We have identified two new kindreds with distinctive clinical syndromes by RFLP analysis identifying the presence of mutant bases in the codons for either amino acid 54 or 55 in one family and 36 or 37 in the other. We have also used a combination of RFLP analysis and amplification by the polymerase chain reaction to demonstrate the presence of an alteration in the TTR gene, corresponding to an amino acid substitution at position 122 in the senile systemic form of amyloid deposition. Each of the mutations results in a somewhat different clinical disease, particularly with respect to age of onset, suggesting that its impact on the physical structure of the molecule has a specific effect on the process of tissue deposition. The investigation of this group of diseases, will allow the analysis of features of proteins responsible for their solubility under physiological conditions as well as providing a model for a therapeutic approach to autosomal dominant diseases in which the abnormal gene product produces tissue dysfunction.

K 305 ANALYSIS OF MUTATIONS IN ONE PATIENT WITH CONGENITAL ADRENAL HYPERPLASIA, Bon-chu Chung and Shiow-Her Chiow, Institute of Moleuclar Biology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C. Congenital adrenal hyperplasia is a common genetic disease. It results from deficient P450c21 (21-hydroxylase) activity which causes virilization and salt loss in severe cases. Patients of the simple virilizing form still retain salts indicating their 21-hydroxylases are partially functional. There are two P450c21 genes in man. The nonfunctional A gene contains a 3.2 kb and the functional B gene a 3.7 kb TaqI fragment. Genomic DNA from one patient with the simple virilizing case of the disease was digested with TaqI and probed with P450c21 cDNA. Two 3.2 and 3.7 kb bands hybridized with equal intensity indicating both alleles of the A and B genes are present. We constructed a partial genomic DNA library by digesting leukocyte DNA from this patient with BamHI, collecting the 13 kb fragments containing the P450c21 genes, and cloning them into the EMBL3 vector. The library was screened with the P450c21 cDNA probe to identify two clones corresponding to the deficient P450c21B genes. The mutant genes have been sequenced and compared with that of the normal gene. Missense mutations which would result in decreased 21-hydroxylase activities in this patient have been identified.

K 306 **CONSTRUCTION OF BACULOVIRUS DERIVATIVES THAT OVERPRODUCE HUMAN α-GALACTOSIDASE A,** George Coppola, Petros Hantzopoulos, and David H. Calhoun, Division of Biochemistry, Department of Chemistry, The City College of New York, Convent Avenue and 138th Street, New York, NY 10031. Fabry disease is an X-linked inborn error of metabolism associated with a deficiency of the lysosomal hydrolase, α-galactosidase A. Previous studies have raised the possibility that enzyme replacement therapy may be beneficial to patients suffering from this condition. We have now constructed derivatives of the baculovirus expression vector that contain a cDNA specific for the precursor form (including the signal peptide) of this enzyme. Catalytically active human α-galactosidase A is produced at levels about 1,000-fold higher than the background, with 70% present in the culture medium and 30% in the cells. This expression system has two advantages over our previously described Escherichia coli K-12 expression system based on the ptrpL1 vector, in that (i) the present construct contains the sequences encoding the signal peptide, and (ii) these eukaryotic cells will carry out N-linked glycosylation during co-translational synthesis. Supported by American Cancer Society grant NP-453B.

K 307 RARE DEFICIENCY ALLELES OF ALPHA$_1$-ANTITRYPSIN
Diane W. Cox, Gail C. Fraizer, Gail D. Billingsley, Todd R. Harrold, Monica Siewertsen, Research Institute, Hospital for Sick Children, Toronto M5G 1X8.
A deficiency of the plasma protease inhibitor α_1-antitrypsin (α1AT), a cause of emphysema and liver disease, is usually associated with the deficiency allele *PI*Z*. However a variety of other alleles which produce the deficiency are being identified. We have studied 222 patients (68 children, 154 adults) with α1AT deficiency. Protein comparisons have been carried out by isoelectric focusing, DNA haplotypes have been studied using 12 polymorphic restriction sites. In addition to *PI*Z*, we identified *PI*Mmalton*, *PI*Mcobalt*, and 4 nonproducing (null or PI*QO) alleles. Based on a population frequency of 0.0122 for *PI*Z*, frequencies for these rare deficiency alleles are $1x10^{-4}$, $2.5x10^{-5}$, and $1.4x10^{-4}$ respectively. All of the rare deficiency alleles can be distinguished from *PI Z* by their DNA haplotype, and most can be distinguished from each other. We have used DNA haplotypes to indicate the presence of new types of null alleles for subsequent sequencing. Our haplotype studies indicate there are at least 4 different null alleles, in addition to 3 previously sequenced (QO*bellingham, QO*granitefalls, and QO*hongkong). We have sequenced *PI*Mmalton*, a type of α1AT similar to Z, which is not secreted normally from the liver. Tertiary structure is apparently altered by the deletion of phenylalanine (amino acid 51 or 52) in one strand within a β sheet, causing a pronounced tendency to self-aggregation. A null allele, QO*bolton, has a deleted C nucleotide in amino acid 362 in exon 5 causing an inframe stop codon at amino acid 373. The mutant site in the two sequenced alleles has been confirmed in genomic DNA in patients and relatives using specific oligonucleotides on PCR amplified DNA. Identification of the spectrum of mutants for α1AT should provide information on requirements for normal production and secretion of hepatocyte glycoproteins.

Biotechnology and Human Genetic Predisposition to Disease

K 308 MOLECULAR CLONING OF THE GENE FOR β-MANNOSIDASE, Norah McCabe and Glyn Dawson. Depts. Pediatrics, Biochemistry, and Molec. Biol., Univ. of Chicago, IL 60637. Cloning of the genes for lysosomal hydrolases has led to enhanced understanding of the type of mutations which lead to human disease. Thus, N-acetyl-β-hexosaminidase deficient mutants can involve deletions, defective RNA splicing, mis-targetted proteins, and unstable proteins, and give rise to a broad spectrum of neurodegenerative disease. We have described β-Mannosidase deficiency, an inherited neurodegenerative storage disease, for which the long-term goal is gene therapy for which animal models are necessary: such a model is goat β-Mannosidosis. We prepared a rabbit polyclonal antibody to purified guinea pig β-Mannosidase (β-Man) which specifically Western-blotted and immunoprecipitated the 100KDa β-man glycoprotein. This antibody was used to screen a λgt11 guinea pig kidney library, and 3 positive 0.5Kb cDNA clones were selected for further study. Authentic β-man clones were verified by generating antibody to the 1089. ITPG-induced fusion protein and showing its ability to immunoprecipitate β-Man. A single, unique, in-frame sequence was obtained, which corresponded to 15KDa of the protein and contained a single N-glycosylation site. The cDNA probe hybridized to guinea pig, human and goat genomic DNA, and the 3Kb human mRNA species detected was sufficient to code for a 100KDa protein. Collaborative studies have localized the β-Man gene to human chromosome 4. It is currently being used to probe both mRNA-positive and mRNA-negative human β-Man mutants, including a unique combined β-mannosidase-sulfamidase deficiency.

K 309 POLYMORPHISMS AT THE HUMAN ALCOHOL- AND ALDEHYDE- DEHYDROGENASE LOCI DETECTED AFTER DNA SEQUENCE AMPLIFICATION, AND DETERMINATION THAT THE INACTIVE *ALDH2* ALLELE IS DOMINANT. Howard J. Edenberg, David W. Crabb, Yiling Xu, Lucinda G. Carr, William F. Bosron and Ting-Kai Li. Departments of Biochemistry, Medicine, and Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223, U.S.A.

Humans are polymorphic at two of the alcohol dehydrogenase (ADH) loci, *ADH2* and *ADH3*, catalyzing the rate-limiting step of ethanol metabolism. Differences in the kinetic properties of the β and γ isozymes encoded at these loci may in part determine an individual's alcohol metabolic rate or susceptibility to alcohol-related pathologies. The acetaldehyde produced by ADH is metabolized by aldehyde dehydrogenases (ALDH) to acetate. A deficiency in the mitochondrial ALDH2 is associated with alcohol-induced flushing in Orientals, and this aversive reaction may play a protective role against alcoholism.

We cloned the human $ADH2^3$ gene and determined the difference between β3 and β1: a change from C to T alters amino acid 369 from Arg in β1 to Cys in β3. The differences between β1 and β2 and between γ1 and γ2 are known. We have used the polymerase chain reaction to amplify the exons that contain sequence differences, and then used either allele-specific probes or RFLPs to identify the alleles. Identification of the *ADH2* and *ADH3* genotypes was unambiguous.

The difference between active and inactive *ALDH2* alleles is a Glu to Lys substitution at residue 487. It had been proposed that only individuals homozygous for the inactive allele, $ALDH2^2$, were deficient in ALDH2 activity. We used PCR and allele-specific oligonucleotides to determine the genotype at this locus. We found that, contrary to earlier proposals, the inactive allele was dominant. This may result from subunit interactions in the tetrameric enzyme.

The ability to distinguish these genotypes with simple and direct tests should be of great utility in studies addressing the role of the different human isozymes in the inter-individual differences in pharmacological and pathological effects of alcohol.

[Supported by PHS R01 AA06460, AA00081 and AA06434, P50 AA7611 and T32 AA07463 from the N.I.A.A.A.]

K 310 IDENTIFICATION OF AN ATYPICAL APO E ALLELE BY PCR AND ASOs, Andrew P. Fellowes, Stephen O. Brennan and Peter M. George, Department of Clinical Biochemistry, Christchurch Hospital, Christchurch, New Zealand.

We have investigated 20 patients with Type III hyperlipidaemia who have the phenotype ε 2/2 by isoelectric focusing, by PCR amplication followed by probing with ASOs (allele specific oligonucleotides) directed against the 158 amino acid site. Nineteen individuals were homozygous for the normal 158 Cys ε2 allele. One previously described individual, heterozygous for $\varepsilon 2/\varepsilon 2_{Christchurch}$ was confirmed as being heterozygous for 158 Cys/158 Arg. This technique allows rapid identification of patients with unusual alleles arising from point mutations in the receptor binding domain of the apo E gene, and direct sequencing of the PCR product allows for rapid characterisation of new alleles.

Biotechnology and Human Genetic Predisposition to Disease

K 311 TESTICULAR DYNEIN ATPase HEAVY CHAIN SHOWS CELL-SPECIFIC EPITOPE AND TRANSCRIPT LOCALIZATION IN BOTH TROUT AND MURINE BRAIN, Anthony T. Garber[1], Nadine A. Seniuk[2], Mayi Arcellana-Panlilio[1], Gilbert A. Schultz[1], Gordon H. Dixon[1], and William G. Tatton[2], Department of Medical Biochemistry, University of Calgary, Calgary[1], and Department of Physiology, University of Toronto, Toronto[2], Canada.
A polyclonal antibody (pab) raised against trout dynein ATPase heavy chains (DYHC) has been used to isolate cDNA clones from a trout testis λgt11 expression library. Northern blot analyses using DYHC cDNA probes revealed that, after testis, brain tissue had the greatest abundance of DYHC transcripts. Southern blot analyses revealed sequence similarities to that for trout DYHC within the genomic DNAs of a number of other vertebrate species. Northern blots of total RNAs prepared from murine brain at various ages showed a progressive increase in two DYHC-related transcripts (3.5 - 4.0 kilobases) relative to ribosomal RNA in aging brains despite decreases in mRNAs for other microtubule-related proteins. Immunocytochemistry for the DYHC pab was used to localize DYHC-related proteins in trout and murine brain. Neuronal somas and their axons, and ciliated ependymal cells showed strong reactions while glial cells were non-reactive. In situ hybridization with biotinylated riboprobes revealed a distinctive perinuclear localization for DYHC-like transcripts in both trout and murine neurons as well as ependymal cells. Transcript density was increased in neurons with long axonal projections as compared to locally ramifying neurons in keeping with a role for dynein-like proteins in fast retrograde axoplasmic transport. These findings indicate that DYHC probes may contribute significantly to studies of the molecular mechanisms of axonal transport in damaged or aging human neurons. (Support by MRC & AHFMR).

K 312 MUTATION DETECTION BY CHEMICAL CLEAVAGE OF PCR AMPLIFIED DNA IN ORNITHINE TRANSCARBAMYLASE DEFICIENCY, Markus Grompe, C.Thomas Caskey, Institute for Molecular Genetics,Baylor College of Medicine, Houston, TX 77030.
Through the use of the polymerase chain reaction it has become possible to amplify mutant DNAs and detect subtle base changes by sequencing. To eliminate the need for sequencing long stretches of DNA we adapted the chemical cleavage method of Cotton et al. (PNAS (1988) 85,4397-4401) to rapidly scan cDNAs for point mutations and to define their location. The cDNA for human ornithine transcarbamylase (OTC) is about 1200 bp long. Two pairs of primers were designed to create overlapping PCR products covering the entire cDNA. Each strand of the wildtype PCR product was endlabelled by further amplification using one of the primers labelled with P32. This probe was used to form a heteroduplex with the PCR product of the mutant cDNA and then treated as in the Cotton protocol. We were able to detect an as yet undescribed mutation in a family with complete OTC deficiency. Upon sequencing the region defined by the chemical cleavage we found a G to A transition in codon 26 involving the last base of exon 1, leading to an Arg to Gln change in the leader peptide of the protein. This base change was confirmed by sequencingamplified genomic DNA. Interestingly, this mutation leads to very low message levels not detectable on Northern blotting.

K 313 EVOLUTIONARY ANALYSIS OF POSITION 57 IN THE HLA-DQ AND DRβ CHAINS IN PRIMATES INDICATES IT IS A BALANCED POLYMORPHISM WITH POSSIBLE CONFORMATIONAL IMPORTANCE. Ulf B. Gyllensten[1,2], Deval A. Lashkari[1], Ines Ezcurra[1] and Henry A. Erlich[1]. 1. Department of Human Genetics, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608, USA. 2. Department of Medical Genetics, Biomedical Center, University of Uppsala, Box 589, S-75123 Uppsala, Sweden.
Genetic susceptibility to several autoimmune diseases e.g. insulin dependent diabetes mellitus (IDDM) and Pemphigus vulgaris (PV) has been shown to be correlated with a neutral amino acid (Ala, Val, Ser) and resistance with a charged amino acid (Asp) at position 57 in the HLA-DQβ chain. The functional significance of position 57 is further suggested by the conservation of this charge polymorphism in all human class II β chains. We have used PCR to examine the nature of the polymorphism in a number of primates including chimpanzee, gorilla, baboon, rhesus, langur and cebus. The polymorphism at this position is highly conserved at all loci examined (DQβ, DRβI and DRβIII) in all the primates, with a very restricted set of residues accepted (Asp, Ser, Ala or Val). The evolutionary maintenance of a balanced polymorphism in all β-chains over at least 10-20 million years suggests that it may be of funtional importance, by possibly affecting the structure of the class II molecule.

K 314 THE CHICKEN DYSTROPHIN cDNA : STRIKING CONSERVATION OF THE C-TERMINAL CODING AND 3' UNTRANSLATED REGIONS BETWEEN MAN AND CHICKEN, Roland Heilig, Catherine Lemaire and Jean-Louis Mandel, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine - 11 rue Humann, 67085 Strasbourg Cédex FRANCE. Dystrophin is a very large muscle protein ($\sim$ 400 kD) the deficiency of which is responsible for Duchenne muscular dystrophy. Its function is unknown at present. In order to know whether different domains of the protein are differentially conserved during evolution, we have cloned and sequenced the chicken dystrophin cDNA. The protein coding sequence has almost the same size than in man. The N-terminal region that resembles the actin binding domain of α actinin, as well as the large spectrin like domain show 80 % and 75 % conservation respectively between chicken and man. In contrast, the carboxy terminal region shows 95 % identity over 627 aa suggesting that it is an important region of interaction with other proteins. Comparison of the amino acid sequence of this C-terminal region to other protein sequences show only marginally significant similarities. Finally we have found a striking conservation of three segments of the 3' untranslated sequence (85 % homology over a total of 920 nucleotides) between chicken and man. These appear also conserved in other mammals. This high conservation is not linked to open reading frames.

K 315 APPLICATION OF THE POLYMERASE CHAIN REACTION (PCR) TECHNIQUE TO THE STUDIES OF THE MOLECULAR BIOLOGY OF GAUCHER'S DISEASE. Nurit Firon[1], Nurit Eyal[1], Edwin H. Kolodny[2], Orly Reiner[1], Ovadia Dagan[1] and Mia Horowitz[1]. Department of Chemical Immunology[1], The Weizmann Institute of Science Israel, and the Eunice Shriver Center for Mental Retardation, 200 Trapelo Road, Waltham, MA 02254, USA[2]
Gaucher disease is a storage disorder characterized by accumulation of glucocerebroside due to a drastic decrease in the enzymatic activity of glucocerebrosidase. So far, four point mutations have been identified among Gaucher patients. Two of the changes are ubiquitous among Gaucher patients while each of the other two mutations have been found in only one patient. We applied the polymerase chain reaction (PCR) technique and the allele specific oligonucleotide (ASO) hybridization for accurate genotyping of three out of the four mutations. Genomic DNAs originating from patients were amplified by the PCR technique. Since there are two human glucocerebrosidase genes of which one is an active gene, we have used a combination of two oligonucleotides that allowed amplification of the active gene alone. Samples of the amplified DNAs on nylon filters were hybridized to oligonucleotides specific for each of the mutations. Since two of the three tested mutations create restriction sites, samples of the amplified DNAs were also tested for the appearance of the new restriction sites. The glucocerebrosidase has an activator protein which promotes the hydrolysis of glucocerebroside by the enzyme. On the basis of a published amino acid sequence of the activator, we synthesized mixed oligonucleotide primers and used them to generate a cDNA probe. This probe was used to isolate a 3.5 kb cDNA from a human fibroblast cDNA library and to characterize two mRNA species which are n3.5 kb in size.

K 316 MOLECULAR CHARACTERIZATION OF CHINESE β-THALASSEMIA MUTATIONS BY AMPLIFIED DNA SAMPLING FROM DRIED BLOOD, Shu-zhen Huang, Xia-di Zhou, Hao Zhu, Zhao-rui Ren and Yitao Zeng, Laboratory of Medical Genetics, Shanghai Children's Hospital, Shanghai, P.R.China
A total of 170 β-thal genes originating from eastern China,southwestern China and southern China (Canton) were amplified directly from dried blood samples to be performed molecular characterization.The amplified sequences included nine Chinese β-thal mutation points. Nine sets of oligonucleotide probes specific for the known Chinese β-thal mutants were hybridized with the amplified sequence.The result revealed:(1)The different types of β-thal mutation were distributed in the different regions,the most commom types in southern China were codons 41-42 -4bp and IVS-II n.654 C→T,constituting nearly 2/3 of the total in this area; the most frequent types in southwestern China were codon 17 and IVS-II n.654 mutations, accounting for 60% of the frequency; and in eastern China,the most prominent mutations were frameshifts at codons 41-42 and 71-72,giving the frequencies as 29.7% and 25.0%,respectively.(2)All the mutants from Canton belonged to the known Chinese mutation types; while 14% of the mutants from eastern China remained unknown.The result is very useful to plan the prenatal diagnosis programs for β-thalassemia. DNA PCR amplification from dried blood provides a rapid and simple method for investigation of the β-thal mutations at large scale.

K 317 COMMON MUTATION IN THE β-SUBUNIT DEFICIENT PROPIONIC ACIDEMIA. Jan P. Kraus, Takahiro Tahara, and Leon E. Rosenberg, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

Propionyl-CoA carboxylase (PCC) is a key enzyme in the catabolic pathway for odd-chain fatty acids, isoleucine, threonine, methionine and valine. The native PCC is an oligomer composed of 6 biotin-containing α-subunits and 6 β-subunits. In humans, inherited deficiency of PCC activity causes life-threatening propionic acidemia. Patients can be classified into 2 major complementation groups, pccA and pccBC, corresponding to the genes encoding the α- and β-subunits, respectively; the BC group is further subdivided into B, C, and BC subgroups. DNA from 3 BC and 5 of 9 C patients, digested with MspI and probed with βPCC cDNA, revealed a unique 2.7 kbp band not observed in blots from 7 pccA patients, 3 pccB patients, or 13 controls. Probing with shorter cDNA probes and performing multiple enzyme digestions made it possible to map the mutation, leading to the appearance of the 2.7 kbp band, to a single MspI site in the coding region of βPCC. The most frequently seen mutations at CpG dinucleotides are C to T or G to A transitions. Therefore, we have prepared oligonucleotides incorporating these changes in the MspI site to test PCR products from control and mutant DNA for differential levels of hybridization. Surprisingly, 2 homozygotes did not show any hybridization with these probes while control DNA did. This means that the MspI site mutation involves more than a single base pair change, most likely a small deletion or insertion. We conclude that 10 out of 30 mutant PCCB alleles have the same mutation at an MspI site in the coding region of the β-subunit. Cloning and sequencing of this mutation is in progress.

K 318 MOLECULAR GENETIC ANALYSIS OF HUMAN AND MOUSE ALBINISM. Byoung S. Kwon, Asifa K. Haq, Mark Wakulchik, Ruth Halaban, Carl Witkop, Chaya Chintamaneni, and Daniel Kestler. Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN; Department of Dermatology, Yale University Medical School, New Haven, CT; Department of Oral Pathology and Genetics, University of Minnesota, Minneapolis, MN.

We have previously isolated and characterized a cDNA for human and mouse tyrosinase. Using the full-length mouse and human cDNA probe, we are investigating the molecular lesions which may be responsible for certain human and mouse albinisms. We have selected for this study human tyrosinase-negative albino families, and Himalayan mouse mutants, which are known to produce temperature-sensitive tyrosinase. We have observed restriction fragment length polymorphisms characteristic to the homozygous albino DNAs in both man and mouse. Tyrosinase cDNA sequences isolated from tyrosinase-negative human albino and Himalayan mouse melanocytes were compared with the normal counterparts. The analysis indicated that frame-shift mutations were responsible for the inactive enzyme.

K 319 STRUCTURE, EVOLUTION, AND MUTATION IN METHYLMALONYL CoA MUTASE (MCM). Fred D. Ledley, Anna-Marie Crane, Ruud Jansen, Sang-Uk Nham, and Mike Wilkemeyer. Howard Hughes Medical Institute, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030.

MCM is a mitochondrial, adenosylcobalamin requiring enzyme whose function in man is to catalyze isomerization of methylmalonyl CoA to succinyl CoA during degradation of various metabolites via propionate. Genetic deficiency of the MCM apoenzyme causes _mut_ methylmalonic acidemia. A cDNA for human MCM has been cloned and sequenced and predicts a protein of 742 amino acids (82,283 Da) comprising a mitochondrial leader sequence of 32 amino acids and a mature protein of 78,489 Da. The homologous murine MCM has also been cloned and exhibits 92% amino acid identity and 88% nucleic acid identity with human MCM in the open reading frame. Homology is apparent among these sequences and _Propionibacterium Shermanii_ MCM reported by Dr. Peter Leadlay, University of Cambridge. _P. Shermanii_ MCM is a heterodimer which catalyzes isomerization of succinyl CoA to methylmalonyl CoA for synthesis of propionate in the ruminant ruminoreticulum. Despite variant quaternary structure and function of the prokaryotic enzyme, there is 22% and 60% identity respectively between the mutA and mutB subunits of _P. Shermanii_ MCM and mature human MCM. MCM cDNA was cloned from five cell lines with the _mut_$^-$ phenotype of decreased apoenzyme activity. Many conservative substitutions (Ile->Val; Phe->Tyr) and two putative mutations (premature termination; Arg->Trp) have been identified and will be characterized by gene transfer. Evolutionary sequence variations which preserve MCM activity and mutations which alter enzyme function should provide insight into the structure and function of MCM.

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K 320 EXPRESSION AND FUNCTIONAL ANALYSIS OF THE HYALURONATE-BINDING REGION OF CARTILAGE PROTEOGLYCAN, Michelle Marks, Kurt Doege, John Hassell and Yoshihiko Yamada, Laboratory of Developmental Biology and Anomalies, NIDR, NIH, Bethesda,MD 20892. Various biochemical studies on the pathogenesis of osteoarthritis indicate both a quantitative and qualitative defect in cartilage proteoglycan and its aggregates. Cartilage proteoglycan aggregates are composed of 2 molecules -proteoglycan monomer and link protein- which interact with each other and with hyaluronic acid. A comparison of the cDNAs which encode cartilage proteoglycan and link protein indicate a common ancestral domain (called Domain 1). The amino terminal Domain 1 of cartilage proteoglycan is subdivided into segments A, B and B' corresponding to the three disulfide loops of link protein. cDNAs encoding portions of Domain 1 were ligated into the pEX vector for expression of Beta-galactosidase fusion proteins in the E. coli strain POP. The cells expressing either Domain 1 in its entirety, subregion A or subregions B plus B' were extracted and the fusion proteins were renatured in the presence of glutathione and protease inhibitors. A solid-phase assay was developed and used to detect HA-binding activity. Fusion proteins containing Domain 1B plus 1B' (the second two disulfide loops of Domain 1) bound to HA with a greater Bmax than did fusion proteins containing only Domain 1A (the first disulfide loop of cartilage proteoglycan). These results suggest that Domains 1B and 1B' are essential for the binding of cartilage proteoglycan to hyaluronic acid.

K 321 CLONING THE HEXOKINASE 1 (HK1) cDNA AND IDENTIFICATION OF PORIN, THE HK RECEPTOR, IN HUMAN MYOCARDIUM, Edward R.B. McCabe[1], Lisa D. Griffin[1], Jeffrey A. Towbin[1], Mikeanne Minter[1], Ferdinando Palmieri[2] and Dieter Brdiczka[3], [1]Baylor College of Medicine, Houston, TX, USA 77030, [2]Universita Di Bari, 70125 Bari, Italy, [3]University of Konstanz, Konstanz, FRG.
HK binds to a specific receptor, porin, on the outer mitochondrial membrane. Porin also binds glycerol kinase (GK) and supplies mitochondrial ATP to HK and GK for phosphorylation of their respective carbohydrate substrates. We previously proposed a model to describe the functional role of this microcompartmented system at the contact points between the inner and outer mitochondrial membranes. There is evidence for developmental and tissue specific regulation of the porin-kinase interactions. We have sequenced 41 amino acids from the N-terminus of 97kD bovine cardiac muscle HK1 and cloned a 109 nucleotide portion of the bovine HK1 gene by mixed oligonucleotide primed amplification of cDNA (MOPAC) using primers of high (256 and 1024 fold) complexity. Two HK1 clones were generated from bovine cardiac muscle cDNA which differed at several nucleotides in the primer sequences but showed identical sequences in the synthesized regions. These clones evidenced 82% nucleotide and 93% amino acid identity with a rat brain HK1 cDNA (Wilson and Schwab, unpublished). We have also demonstrated the presence of the HK1 receptor, porin, in human myocardium. Monospecific, polyclonal antibody preparations to bovine heart and rat liver porin showed the presence of an appropriately sized (34kD) protein which cosedimented with the mitochondrial fraction. We are currently engaged in cloning full length HK1 and porin cDNAs in order to investigate the molecular biology of metabolic microcompartmentation involving the porin-kinase system.

K 322 Isolation of Mouse Mutants with Heritable Hyperphenylalaninemia, McDonald, J.D.*, Bode, V.C.+, Dove, W.F.*, Thompson, K.*, and Shedlovsky, A.*, *McArdle Laboratory and Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, +Division of Biology, Kansas State University, Manhattan, KS 66502. In order to produce mouse models for human phenylketonuria (PKU) and hyperphenylalaninemia, we have screened the progeny of ethylnitrosourea-treated males for defects in phenylalanine (PHE) catabolism. Four autosomal recessive mutations have been identified, each resulting in the reduced ability to clear a PHE challenge. The first, *hph-1* (hyperphenylalaninemia-1), has been mapped to chromosome 14 and biochemically characterized as a deficiency in liver GTP-cyclohydrolase activity. The subsequent mutations, *hph-2*, *-4*, and *-5* are currently being characterized. Based on the results of genetic complementation and recombination analysis, there is evidence that *hph-2* and *hph-4* may be allelic. Animals homozygous for the *hph-5* mutation exhibit a severe deficiency of liver PHE hydroxylase activity. Thus, in its biochemical phenotype, the HPH-5 mouse strain appears to fulfill our goal of providing a mouse model for human PKU.

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K 323 INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN (IRBP). J. Nickerson, D. Borst, T. T. Redmond, J-S. Si, and G. Chader, NEI, NIH, Bethesda, MD 20892.

IRBP is a large glycolipoprotein (about 140 kDa) found in high concentration between the retinal photoreceptor cells and the underlying pigment epithelium in the vertebrate eye. IRBP likely plays a key role in the transport of retinoids in this extracellular space. We have cloned bovine and mouse IRBP genes and bovine and human cDNAs. The bovine gene sequence contains four exons and three introns. The gene is 11.6 kb in size. Two of the exons, the first and the fourth, are large (3.2 and 2.4 kb, respectively). The introns are each 1.5 to 2.2 kb. We have isolated an IRBP genomic clone from a human chromosome 10-specific library, confirming the locus assignment (10q11.2), and its gene structure similarity to that of cow. The IRBP mRNA is large, 4.3 kb in human and 6.0 kb in bovine excluding poly(A) tails. The major difference in the mRNA structures is the insertion/deletion of a 1.6 kb segment in the 3' untranslated region. There is a four-fold repeat structure in the IRBP protein sequence with about 300 amino acids per repeat, and 30-40% identity between any two repeats. The boundaries of the four protein repeats do not correspond to the positions of the three introns in the gene. All 3 introns are in the fourth repeat. The gene structure suggests that some ancestral introns were lost early in the quadruplication process. Partial DNA and deduced protein sequences from the mouse gene are quite similar to the bovine and human sequences, suggesting an essential role for IRBP in the visual process. The deduced IRBP protein sequences have been helpful in the analysis of an inflammatory disease, uveitis, and its animal model, experimental autoimmune uveitis (EAU). Short peptides based on the protein sequence and IRBP-β-gal fusion proteins cause EAU.

K 324 A LABORATORY ORIENTED WORKSHOP FOR TEACHING THE METHODOLOGY FOR DNA DIAGNOSIS OF HUMAN GENETIC DISEASE - WILLIAM C. NIERMAN AND DONNA R. MAGLOTT, AMERICAN TYPE CULTURE COLLECTION, ROCKVILLE, MD 20852

A laboratory intensive workshop for teaching approaches to diagnosing human genetic disease by molecular biological techniques has been developed and offered at the ATCC. The laboratory exercises, directed toward clinicians, included cystic fibrosis by analysis of restriction fragment length polymorphisms, detection of deletions in the Duchenne's muscular dystrophy locus using cDNA probes, detection of gene amplification in breast tumor cells, and detection of site mutations using the polymerase chain reaction and dot blotting with oligonucleotide probes. The focus has been on methods and not diseases. Both direct and indirect detection methods were covered in the laboratory setting. Our experience in the first offering of the course will be presented.

K 325 **LOCALIZATION OF THE GENE FOR AUTOSOMAL DOMINANT, HLA-LINKED SPINOCEREBELLAR ATAXIA (SCA) WITHIN A SMALL REGION OF CHROMOSOME 6**, Harry T. Orr[1], Larry Schut[2], Patricia Wilkie[1], Lisa Duvick[1], Stephen S. Rich[1], [1]Department of Laboratory Medicine and Pathology and Institute of Human Genetics, University of Minnesota and [2]GRECC, V.A. Medical Center, Minneapolis, MN 55455.

Inherited spinocerebellar ataxias (SCA) are progressively degenerative neurological diseases. The primary site of degeneration is the cerebellar cortex; in particular, Purkinje neurons. A large, seven-generation kindred having an autosomal dominant, HLA-linked form of SCA is being analyzed to determine the precise location of the SCA locus on chromosome 6. Pairwise and multipoint linkage analysis using HLA-A, SCA, coagulation factor XIIIa (F13A), and the DNA marker 12.3.6 resulted in a most favored order of:

HLA-A-(8 cM)-p12.3.6-(10 cM)-SCA-(10 cM)-F13A-6pter

The odds in favor of this order over the next most likely order (SCA centromeric to HLA-A) are 50,000:1. A second large kindred with clinically similar HLA-linked, autosomal dominant SCA is currently being collected and studied with these markers. Initial analyses with HLA-A indicate SCA is linked at a distance of 23 cM with 3.6 lod score. No evidence in favor of linkage heterogeneity has been detected with these two large kindreds.

K 326 **MOLECULAR CLONING AND SEQUENCE ANALYSIS OF A HUMAN SERUM TRANSFERRIN cDNA,**
Paul R. Rosteck, Jr., Bradley S. DeHoff, Jeffrey L. Larson, and Charles L. Hershberger, Lilly Research Laboratories, A Division of Eli Lilly and Company, Indianapolis, IN 46285
A cDNA library constructed from polyA+ mRNA from human liver was screened with a synthetic 45-mer oligodeoxyribonucleotide probe corresponding to a portion of exons 11 and 12 of the transferrin gene. Twelve positive clones were identified and the plasmid containing the longest insert, pHDM99, was selected for further study. The complete nucleotide sequence of the insert was determined by oligodeoxyribonucleotide primer-directed gene walking . The 2,340 base pair insert encodes the complete human transferrin protein from amino acid -19 of the signal peptide through the COOH-terminus of the mature protein. In addition, the clone contains a 3' non-translated region of 166 nucleotides and a 5' non-coding segment of 46 nucleotides. The deduced amino acid sequence agrees with previously determined transferrin sequences except at position 310 of the mature protein where aspartate is encoded in place of the previously determined asparagine residue. Nine additional nucleotide differences are present in the transferrin coding sequence but all are in degenerate third positions of codons and do not result in additional amino acid changes. We propose that this clone encodes a new member of the polymorphic transferrin gene family.

K 327 ISOLATED GROWTH HORMONE DEFICIENCY: SEARCH FOR THE MOLECULAR BASIS.
Fred Schaefer, Jennifer Swartz-Boyd, Elaine Mardis, Sara Wiley, Don Wilson and Bruce Roe, Chapman Research Inst., Children's Med. Ctr., Tulsa, and, Univ. of Okla. Norman, OK
Our laboratory is attempting to determine the molecular basis of isolated growth hormone deficiency (IGHD). In one of our studies, the incidence of IGHD1A (a deletion within the growth hormone gene) was studied by Southern techniques. To date, 52 patients from 51 different families from Arkansas, Oklahoma and Texas have been studied; no deletions of the hGH 3.8 kb band have been found. These results indicate that the incidence of IGHD 1A in midwest population is less than 5% ($p<0.05$) of the total IGHD population and 95% or more suffer from a defect which has not yet been described.

In search of another cause of IGHD, growth hormone releasing factor (GHRF) is also being studied. The DNA of IGHD patients and controls is being examined using polymerase chain reaction (PCR) techniques and direct sequencing. The 470 bp regulatory region of GHRF including the TATA and CAAT box, mRNA initiation site and 77bp first exon has been studied first. The DNA of 40 IGHD patients and 26 controls has been amplified and electrophoresed on a 2% agarose gel and no significant differences in size have been observed. To date, 27 IGHD Patients and 14 controls have been further evaluated by sequencing. There is a strong stop obscurring part of the sequence, however, all the known regulatory elements are clearly sequenced and contain no point mutations.

These results indicate that neither major insertions or deletions nor point mutations of the regulatory region of GHRF are major causes of IGHD. Studies of the last 2 exons of GHRF do not detect a gross deletion or insertion. Studies to detect point mutations are underway as are studies of other potentially defective genes in the GH cascade.

K 328 CIS-ACTING REGULATORY ELEMENTS OF THE MOUSE ORNITHINE TRANSCARBAMYLASE GENE,
Steven E. Scherer, William J. Craigen, Gabor Veres and C. Thomas Caskey, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030
The urea cycle enzyme ornithine transcarbamylase (OTC) is expressed primarily in liver, and to a lesser extent, small bowel. To define the cis-acting regulatory elements responsible for tissue-specific expression, we have examined DNA sequences upstream of the transcription initiation site. A 735 base pair (bp) fragment is sufficient to direct the expression of the chloramphenicol acetyl transferase (CAT) reporter gene in Hep G2 human hepatoma cells, but not NIH 3T3 cells. In addition, a 4kbp 5' fragment containing this region promotes high level hepatic and bowel CAT expression in transgenic mice. Deletion analysis of this promoter region demonstrates that sequences from -203 to the transcription start site are critical for high level expression of OTC. Sequences from -491 to -203 also contribute to promoter activity, but to a lesser extent. Gel retardation assays have identified DNA fragments from -428 to -278 and from -103 to the transcription start site that are specically bound by proteins from a mouse liver nuclear extract. Taken together, the deletion studies and gel retardation assays suggest that at least two regions of the promoter are required for tissue-specific expression of OTC. We are currently employing DNase I footprinting analysis using extracts derived from a variety of tissues to further define the DNA sequence specificity of the OTC cis-acting regulatory elements.

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K 329 MOLECULAR STUDIES OF THE TYROSINASE GENE IN HUMAN OCULOCUTANEOUS ALBINISM. Richard A. Spritz*, Kathleen Strunk*, William S. Oetting+, and Richard A. King+. *Dept. of Medical Genetics, Univ. of Wisconsin, Madison, WI & +Inst. of Human Genetics, Univ. of Minnesota, Minneapolis, MN.

The oculocutaneous albinism (OCA) syndromes present with deficient synthesis of melanin pigment in skin, hair, and eyes, associated with altered development of the central visual system. A number of different OCA syndromes are distinguished by clinical, enzymologic (tyrosinase), and genetic criteria; however, the relationships among these disorders are unclear. We have used a mouse tyrosinase cDNA to identify an RFLP within the human tyrosinase gene on chromosome 11q, and have begun to use this RFLP as a linkage marker to define the molecular relationships among different types of OCA.

In type IA (classic autosomal recessive tyrosinase-negative) OCA, we have established linkage between OCA and the tyrosinase gene in a number of families. Non-obligate heterozygosity was established by hairbulb tyrosinase activity analysis. We have found no tyrosinase gene deletions in type IA OCA individuals, including three with mental retardation. Therefore, type IA OCA most likely results from single-base substitutions in the tyrosinase gene that interfere with tyrosinase biosynthesis or function. Furthermore, type IA OCA does not appear to result from one common mutation, since both RFLP alleles were found among the type IA OCA individuals. Analyses of DNA sequences of normal and type IA OCA tyrosinase genes are in progress.

We have excluded linkage between the tyrosinase gene and autosomal dominant OCA in one family, showing that this mild type of OCA does not result from the heterozygous effects of a tyrosinase gene mutation.

We are currently extending these studies to other types of OCA that may result from mutations of the tyrosinase gene, including IB (yellow) and type III (minimal pigment) OCA. These studies should determine if there is a multiallelic system at the human tyrosinase locus that is similar to that at the *c* locus in the mouse. These investigations should also result in improved classification, carrier detection, and perhaps prenatal diagnosis of several types of oculocutaneous albinism.

K 330 AMPLIFICATION OF DNA LINKED TO THE HUNTINGTON DISEASE LOCUS AND PROBLEMS WITH THE SUBSEQUENT RESTRICTION ENZYME ANALYSIS OF THREE POLYMORPHIC SITES, Patricia M. Stapleton, Department of Cellular and Molecular Biology, University of Auckland, Auckland, New Zealand.

Huntington disease (HD) is a late onset condition determined by a single dominant locus which is linked to several restriction enzyme sites that give diagnostic restriction fragment length polymorphisms (RFLPs). The reliability and rapidity with which the disease state can be diagnosed should be enhanced by the polymerase chain reaction (PCR).

The DNA surrounding each of three sites which produce RFLPs that are linked to the HD locus was sequenced to provide the necessary information for the amplification of these sites using the PCR (1). The three regions were amplified successfully. However, the results from the subsequent analyses of the amplified products by restriction enzyme digestion were unreliable. The presence of non-target sequences hindered the detection of a person's genotype at two of the sites and masked the true genotype of a person at the third site.

(1) Stapleton, P.M. (1988). Nucleic Acids Research 16, 2735.

K 331 HEMOPHILIA B RESULTING FROM *DE NOVO* INSERTION OF AN ALU SEQUENCE. Michel VIDAUD, Dominique VIDAUD, Virginie SIGURET, Jean Maurice LAVERGNE and Michel GOOSSENS, INSERM U.91, Hôpital Henri Mondor, 94010 Créteil, and INSERM U.143, Hôpital de Bicetre, 94275 Paris. Alu elements are the most abundant short interspersed related sequences (SINEs) in the human genome, and transpositions are thought to account for their dispersion and high copy number. We report here the *de novo* insertion of an Alu sequence into the exon V of the blood coagulation factor IX gene in a patient with severe hemophilia B. This sequence displays all the characteristics of the Alu family elements and this observation is, to our knowledge, the first description of a mutational insertion of an Alu sequence as a cause of a human disease. It is likely that this Alu insertion, which is also found in one of the mother's Factor IX genes, occured in the grand father sperm or after fertilization. Our results indicate that Alu sequences can actually be dispersed in human cells presumably through an RNA intermediate and further suggest that retrotransposition continues to shape the human genome.

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K 332 A MOLECULAR ANALYSIS OF HUMAN ARGININOSUCCINATE (ASAL) LYASE DEFICIENCY.
David C. Walker, Deborah A. McCloskey, Louise R. Simard and Roderick R. McInnes, Hospital for Sick Children, Univ. of Toronto, Toronto, Canada, M5G 1X8
Argininosuccinic aciduria is a clinically heterogeneous, autosomal recessive, inborn error of the urea cycle. We have previously demonstrated frequent intragenic complementation in a group of 28 mutants that have substantial genetic heterogeneity at a single locus (presumably the ASAL structural gene). Most patients are likely to be genetic compounds of two rare alleles. Northern blot analysis using the 1585 bp human ASAL cDNA shows that 25 mutants have normal size and quantity of mRNA, 1 strain has reduced levels, and 2 others have no detectable message. Southern blots (Eco RI and Bgl I digests) reveal no major alterations in any mutant gene. These results suggest that the majority of the alleles have small or single base pair changes, and identify several interesting classes of mutants: 1) two strains that complement frequently (suggesting normal subunit interaction) and that may contain mutations affecting the active site, 2) two pairs of mutants that on complementation have unusually high restoration of enzyme activity, and 3) many mutants that produce either an unstable monomer or only trace amounts of it. We have used the polymerase chain reaction to amplify and sequence the cDNA transcribed from mutant fibroblast ASAL mRNA (abundance ~0.01%), in three overlapping ~500bp segments. RNAse A cleavage and sequence analysis of the PCR product will identify the mutations. Expression studies show that our normal cDNA expresses enzyme activity in ASAL-deficient CHO cells and can be used as a sequence reference in mutant analysis. A comprehensive characterization of the single bp mutations at this locus is feasible using this approach, and in conjunction with the expression of mutant proteins, we will be able to determine the genetic basis of the intragenic complementation, mutant protein phenotypes, and clinical heterogeneity in this disorder.

K 333 CLOSING IN ON THE MYOTONIC DYSTROPHY LOCUS AT 19q: Use of V(ariable) S(imple) S(equence) M(otif) Markers.
Bert Smeets, Han Brunner, Marga Coerwinkel-Driessen, Rosella Hermens, Hans-Hilger Ropers and Bé Wieringa. Dept. of Human Genetics University Nijmegen P.O. Box 9101 6500 HB Nijmegen The Netherlands.

Myotonic dystrophy is a frequent autosomal dominant disorder characterised by a variety of symptoms among which myotonia, muscular atrophy, cataracts and behavioural changes are the most prominent. By use of two- and multipoint linkage analysis we have located the DM gene on 19q, close to and just distal of the apolipoprotein C2/C1/E cluster (θ=3, Zmax >30) and the CKMM gene (θ=2, Zmax>24) at q13.2 and proximal of the pEFD4.2 VNTR-marker at q13.3. Based on physical evidence the chromosomal interval thought to contain the DM gene currently is estimated to span at most 4 Mbp. With the use of CHEF- and FIGE-electrophoresis we have mapped several unique DNA elements isolated from this interval with respect to the CKMM-gene as a central demarcation point. Newly isolated probes pR83B18, pR86B18 and pR88B18 are just proximal of CKMM and close to the apoC2/C1/E cluster; the ERCC1 (repair-) gene maps within 200 kbp distal of CKMM.

To optimize the use of our family material and to further narrow down the location of DM within cross-over borders nearby we have used a novel class of highly informative markers based on the variable simple sequence motifs (VSSM's, composed of di-and trinucleotide repeats) which are ubiquitously dispersed throughout the human genome. Two q13.2 specific VSSM's, composed of [dC-dA]n.[dG-dA]n blocks, one located in the first intron of the apoC2 gene and another close to the ERCC1 gene were identified and analysed into detail using PCR amplification, high resolution gel electrophoresis and DNA sequencing. In the population a high degree of allelic variation with alleles varying in length n of the CA or GA blocks was found and even within individuals somatic microheterogeneity was observed. As yet no cross-overs between the ERCC1-VSSM and DM were found. We further demonstrate that both VSSM-markers can be used as reliable genetic markers for DM-diagnosis. Moreover, our results suggest that study of the many thousands of structural similar VSSM-markers in the human genome can be employed to allow widespread detection of heritable disorders.

K 334 CORRECTION OF PATHOLOGY IN PRIMARY CULTURES OF MUCOPOLYSACCHARIDOSIS VII CELLS BY RETROVIRAL VECTOR TRANSFER OF A BETA-GLUCURONIDASE GENE
JH Wolfe*, EH Schuchman#, LE Stramm*, EA Uhlenburg*, ME Haskins*, G Aguirre*, DF Patterson*, RR Desnick#, E Gilboa+. *Univ Penna Vet Med, Phila, PA; +Sloan-Kettering Inst, New York, NY; #Mt. Sinai Med, New York, NY.
Beta-glucuronidase (b-gluc) deficient mucopolysaccharidosis (MPS) VII (Sly disease) occurs in humans and dogs. The lysosomes of b-gluc deficient cells accumulate specific glycosaminoglycans (GAGs). The similarity of the human and canine diseases make MPS VII dogs an attractive model system for somatic cell gene therapy. Retroviral vectors were constructed using the N2 vector containing minigenes of the rat b-gluc cDNA driven by either the SV40 or TK promoter. Vector transfer of the b-gluc gene restored b-gluc enzymatic activity in human MPS VII fibroblast cell lines and canine MPS VII bone marrow cells. In addition to the standard quantitative assay, b-gluc activity was visualized in single cells using a histochemical staining technique. Vector transfer of the b-gluc gene into primary cultures of canine MPS VII retinal pigment epithelial (RPE) cells restored b-gluc enzymatic activity and degraded the accumulated $^{35}SO_4$-labelled GAGs to the levels seen in RPE cells from a genetically normal dog. These results indicate that the rat enzyme is properly processed, targeted to lysosomes, and catalytically active towards the accumulated natural substrate. Based on these studies, in vivo gene transfer studies have been started.

K 335 GENE DIAGNOSIS OF HEMOGLOBINOPATHY IN CHINESE BY AMPLIFIED DNA, Yi-tao Zeng, Shu-zhen Huang, Xia-di Zhou, Hao Zhu and Zhao-rui Ren, Laboratory of Medical Genetics, Shanghai Children's Hospital, Shanghai, the People's Republic of China
Here we report the application of DNA PCR amplification on the prenatal diagnosis of thalassemia syndromes and on detection of β^S and β^D Punjab genes.DNA amplification was performed on lysed amniotic fluid cells or chorionic villus samples and on DNA sampling from dried blood without prior DNA extraction. α-thalassemia was prenatally diagnosed by direct analysis of amplified fetal target sequences on gel electrophoresis; and β-thalassemia was predicted by Hgi AI RLFP linkage analysis with amplified β-globin DNA. Sickle cell gene was detected by Mst II mapping of the amplified β-globin DNA directly on the electrophoretic gels. For identification of Hb D Punjab (β121 $\frac{GAA \rightarrow CAA}{Glu \rightarrow Gln}$),the most commom variant in western China, the primers were designed and synthesized to amplify a 144bp fragment β-globin gene, including codons 121 (GAA) and 122 (TTC). The 5'GAATTC-3' was just an Eco RI recognition site. So Hb D Punjab gene could be easily identified by Eco RI mapping of the 144bp amplified sequence,because of a single base change at codon 121. The analyses of the amplified DNA does not require radioactive DNA probe and southern hybridization. The total procedure can be completed within five hours. This simple method provides a powerful tool for gene diagnosis of hemoglobinopathy or other genetic diseases.

DNA Repair, Drug Design, Genetic Engineering

K 400 **EXPRESSION OF BIOLOGICALLY ACTIVE HUMAN PLASMA PROTEINS IN ESCHERICHIA COLI**
Egon Amann, Karl-Josef Abel, Mathias Grote, Jürgen Römisch, Ulrich Grundmann, Hiroshi Okazaki# and H.A. Küpper, Department of Molecular Biology Behringwerke AG, Postfach 1140, D-3550 Marburg, F.R.G. and #Hoechst Japan Limited, Pharma R&D Division, 3-2, Minamidai 1-Chome, Kawagoe, Saitama 350, Japan. **We have developed novel plasmid expression vectors suitable for the regulated cytoplasmatic expression of unfused proteins in Escherichia coli (GENE 69 (1988) 301-315). These vectors have been employed for the synthesis of i) the human plasma and placental protein FXIIIa (PNAS 83 (1986) 8024-8028), ii) the anticoagulant protein PP4 (PNAS 85 (1988) 3708-3710) and iii) the anticoagulant protein PP4X (Behring Inst. Mitt., 82 (1988) 59-67). FXIIIa is a protein of 731 amino acids and acts as a fibrinoligase in the final stages of the blood coagulation process. PP4 (320 amino acids) and PP4X (321 amino acids) both belong to the phospholipase A2 inhibitor family (collectively called lipocortins) and share a high degree of homology. All three proteins could be expressed to high levels in E.coli cytoplasmatically (1-2 g of protein/l culture under fermentation conditions). However, the protein behave remakably different: Whereas most of the FXIIIa protein is expressed in a denatured form as inclusion bodies, PP4 and PP4X are soluble and can be recovered in biologically active form after cell breakage. After removal of the FXIIIa inclusion bodies from cellular lysates, an additional, soluble form of FXIIIa is observed in the supernatants, which is biologically active. Sequence determinations at the aminoterminus of FXIIIa and PP4 revealed the absence of the initiator methionines. Thus, the recombinant proteins diplay the identical amino acid sequences compared to the proteins isolated from the natural sources.**

K 401 **GENETIC LINKAGE STUDIES IN FANCONI ANEMIA,** **Arleen D. Auerbach, V.S. Venkatraj, William R. Mann, Rita Ghosh, Liu Qian, Drew Olsen, Stephanie L. Sherman, The Rockefeller Univ., New York, NY and Emory Univ. School of Medicine, Atlanta GA.**
Through the International Fanconi Anemia Registry, maintained by us at Rockefeller, we have identified 25 families that are informative for linkage studies: 13 with 2 or more affected offspring, 9 with 1 or more offspring from consanguineous marriages and 3 with multiple affected children in collateral, or more distant sibships. We defined as affected all individuals demonstrating hypersensitivity to the DNA crosslinking agent diepoxybutane. The potential for detecting linkage in the pedigrees is demonstrated by an ELOD of >5.0 or 1.25 for markers at 10% or 30% recombination respectively. Chromosome 7q is a candidate region for FA, as patients are predisposed to AML with $7q^-$ clones. The following lod scores were obtained, using the computer program LIPED.

		Lod at Recombination Fraction (θ) of				
Locus	N^a	.00	.10	.20	.30	.40
D7S87 (CRI-pS94)	12	-∞	-3.39	-1.28	-.45	-.10
D7S72 (CRI-pS3)	16	-∞	-1.94	-.55	-.19	-.10
D7S104 (CRI-pS194)	11	-∞	-2.67	-.69	-.12	.00

a: Number of informative families

As θ_{max} was 0.5 for each marker, we can exclude most of the long arm of chromosome 7 as apossible location for the FA gene. Additional markers spanning other chromosomes will be studied.

K 402 MISMATCH REPAIR IN EXTRACTS OF XENOPUS EGGS: BASE SPECIFICITY AND EFFICIENCY OF REPAIR FOR DIFFERENT MISMATCHES. Peter Brooks, Christiane Dohet, Isabelle Varlet, Jan Filipski and Miroslav Radman, Institut Jacques Monod, CNRS, 2 place Jussieu, 75251 PARIS Cedex 05, France.

Mismatch repair in extracts of *Xenopus* eggs has been characterized. Repair was detected by a physical assay and shown to be specific by mismatch-localized DNA repair synthesis. In addition, mismatch-localized synthesis in non-repaired molecules may represent intermediates in the repair process. The repair synthesis involves kilobase stretches and sensitivity of repair to aphidicolin has been observed. Nicks placed at a distance of a few hundred base pairs from the mismatch resulted in stimulation of both repair and repair synthesis specifically on the strand bearing the chain breaks. A similar distribution of DNA synthesis was not observed with nicked homoduplex DNA. The spectrum of repair efficiency and specificity for the 12 single base-pair mismatches has been determined by using a set of heteroduplex substrates with the mismatches placed in four overlapping restriction sites. The choice and positions of these sites enabled the formation of all possible mismatches without any other sequence changes. The cassettes have been placed in different sequence contexts including a eukaryotic promoter sequence and an IgG variable region. The effect of these environments on mismatch repair efficiency is under investigation.

K 403 MOLECULAR CLONING OF cDNAs WHICH CONFER UV-RESISTANT PHENOTYPES UPON XERODERMA PIGMENTOSUM GROUPS C AND D CELL LINES. D. Canaani, T. Teitz, T. Naiman, D. Eli, M. Penner, M. Stark and M. Bakhanashvili, Department of Biochemistry, Tel Aviv University, Ramat Aviv 69978, Israel.

A human cDNA clone library constructed in a mammalian expression vector has been previously used to complement the UV-sensitive phenotype of a xeroderma pigmentosum group C cell line (Teitz et al., Proc. Natl. Acad. Sci. USA 84: 8801-8804; 1987). The primary transformants selected for UV-resistance also acquired normal levels of DNA repair. Using a similar approach we have recently been able to partially complement the UV sensitivity of a xeroderma pigmentosum cell line belonging to complementation group D. The rescue and molecular cloning of two partial length cDNAs which confer, respectively, a UV-resistant phenotype upon XP-C and XP-D cell lines, will be described.

K 404 POTENTIAL PROBLEMS IN SOMATIC GENE REPLACEMENT THERAPY - P. L. Chang, E. Johannson, R. M. Lafrenie, J. P. Capone, W. Orr and G. M. Brown. Departments of Pediatrics, Medical Sciences, Biochemistry and Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Transkaryotic implantation is an alternate approach to gene replacement therapy in which fibroblasts transfected with the desired gene are implanted to deliver the gene product. To evaluate its potential applications in humans, and to avoid using potentially oncogenic retroviral vectors, we transfected primary fibroblasts established from rat skin biopsies by calcium phosphate precipitation with a plasmid DNA encoding the human growth hormone gene and G418-resistance. Clonal fibroblast cell strains selected for G418-resistance and human growth hormone expression were implanted either intraperitoneally, or subcutaneously or intra-muscularly into the original donors. Contrary to previous observation, human growth hormone was detected in the host circulation over an extended period of 6-8 months but at highly fluctuating levels, sometimes reaching baseline values. Extremely high titres of antibodies against human growth hormone were detected, probably accounting for the apparent early cessation of gene expression noted before. One of the autologously implanted animals developed fibrosarcomas in the intraperitoneal cavity. Human growth hormone was detected in the tumor tissue with immunofluorescence and human growth hormone sequence was detected with Southern blotting in the tumor cell lines, thus indicating that the tumors were derived from the implanted fibroblasts. Hence, antigenic responses from the host and potential tumorogenicity of the implants even without the use of retroviral vectors must be assessed in any gene replacement therapy.

K 405 CIS-PLATINUM RESISTANCE IN HUMAN CELLS, Chuck C.-K. Chao[1], Y.L. Lee[1], P.W. Chen[1] and Sue Lin-Chao[2], [1]Tumor Biology Laboratory, Chang Gung Medical College, Taiwan 33333 and [2]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305.
Development of resistance to cis-platinum (cis-Pt) was achieved in human cervix carcinoma HeLa cells. Following continuous selection pressure, a 10-fold increase in resistance relative to the parent line was observed. At this time, further increase in selection pressure resulted in cell death, while removal of drug led to rapid loss of resistance. In the presence of intermittent low concentrations of cis-Pt, resistance has been maintained in excess of 6 months. There is similar resistance to drug of cells established by stepwise selection strategy. Experiments currently are in progress to investigate the molecular mechanism of cis-Pt resistance. For example, cellular ability in repairing DNA damage was measured by *in vivo* transient CAT assay or *in vitro* analysis. For comparison, we have examined VA13, a repair-competent, and XPA, a repair-defective cell lines. The results indicate significant increase in DNA repair in cis-Pt resistant cell lines.

K 406 **GENETIC MAPPING OF THE HUMAN CHROMOSOME 11Q22-23 REGION: IMPLICATIONS For FURTHER LOCALIZATION Of The GENE For ATAXIA-TELANGIECTASIA.** Patrick Charmley, Ozden Sanal, Umah Malhotra*, Shan Wei, Tatiana Faround, Patrick Concannon*, and Richard A. Gatti. UCLA School of Medicine, Los Angeles, CA 90024, and *Virginia Mason Research Center, Seattle, WA 98101.

Human chromosomal region 11q22-23 is of interest because of the report by Gatti et al (Nature, in press) which describes the localization of a gene for the disease ataxia-telangiectasia. This disease was initially mapped to 11q22-23 by an RFLP detected with the gene for thy-1. However, there are other candidate genes in this region, e.g. CD3-delta, CD3-epsilon, CD3-gamma, N-CAM, and the proto-oncogene ets-1. The apolipoprotein gene complex A1-C3-A4 and several RFLP's detected with arbitrary DNA sequences also map to this region. This relative abundance of genetic markers suggests the importance for fine mapping of 11q22-23 for the purpose of more precisely localizing the ataxia gene. We have therefore identified novel RFLP's detected with the above mentioned structural genes, and we have used those RFLP's with the 40 CEPH index families in order to construct a detailed genetic map of this region. By this segregation analysis, we have 1) determined the order of these DNA markers, 2) generated haplotypes for the 11q22-23 region, and 3) searched for linkage disequilibrium between the markers. These analyses will allow us to more precisely, and efficiently, localize the gene(s) for ataxia-telangiectasia.

K 407 THE RECOGNITION OF DAMAGED DNA IS DEFECTIVE IN XERODERMA PIGMENTOSUM GROUP E, Gilbert Chu and Elaine Chang, Department of Medicine, Stanford University Medical Center, Stanford, CA 94305. Individuals with the autosomal recessive disease xeroderma pigmentosum (XP) suffer from photosensitivity and cancer of the skin. Their cells share the phenotype of defective DNA repair, but fall into at least nine genetic complementation groups, as defined by cell fusions. Thus, DNA repair in humans involves multiple gene products.

We have extended the gel electrophoresis binding assay to identify proteins that bind to damaged DNA, independently of DNA sequence. Extracts from HeLa cells contain binding activity for DNA damaged by ultraviolet radiation or the antitumor drug cisplatin and for single stranded DNA. The activity is localized to the nucleus; present in XP cells from seven complementation groups; but notably absent from XP group E cells. Binding activity appears as protein-DNA complexes of two different electrophoretic mobilities. Chromatographic fractionation is underway to see if a single protein is involved in the binding activity. In conclusion, we have identified at least one protein factor that appears to participate in a versatile DNA repair pathway at the stage of binding and recognition.

K 408 ANALYSIS OF THE INDUCTION AND REPAIR OF DOUBLE STRANDED DNA BREAKS IN MAMMALIAN CELLS BY ASYMMETRIC FIELD INVERSION GEL ELECTROPHORESIS, Nicholas Denko, Penny Jeggo, Thomas D. Stamato, The Wistar Institute, Philadelphia, PA 19104
Asymmetric field inversion gel electrophoresis (AFIGE) is a variation of pulsed field gel electrophoresis capable of resolving DNA fragments of >9 mbp. We have devised a technique using AFIGE to measure frank double stranded DNA breaks (dsbs) induced by ionizing radiation at doses as low as 250 rads. Cells are uniformly labeled with ^{14}C thymidine, suspended in warm (37°C) agarose which is allowed to gel. Cells in agarose plugs are irradiated on ice and either lysed immediately, or allowed to repair in pre-warmed media for various periods of time after which they are lysed and the DNA purified while still in the agarose plug. The plugs are loaded into wells of an 0.8% agarose gel and subjected to AFIGE electrophoresis. The fraction of DNA remaining in the plug is determined by counting radioactivity in two agarose fragments. One fragment contains DNA which has migrated into the lane and the other contains DNA remaining in the plug. DNA from γ-irradiated cells is released into the lane in a dose dependent response that is log-linear up to 4-5 krads. Using this technique, we have examined the repair kinetics of wild type and mutant CHO cells and find that X-ray sensitive CHO lines XR-1, xrsl, and xrs5 are deficient in the repair of frank dsbs induced by γ-rays.

K 409 CHROMATIN STRUCTURE REGULATES BASE AND NUCLEOTIDE EXCISION REPAIR, Klaus Erixon, Medical Radiobiology, Karolinska Institute, S-104 01 Stockholm, Sweden.
Cells respond to UV-mimetic damage by the process of **nucleotide exision repair**, giving rise to long repair patches. Incision was followed by the accumulation of single-strand breaks (SSB) in the presence of DNA polymeras inhibitors, like aphidicoline (ApC). Cells from individuals with the disease xeroderma pigmentosum (xp) are deficient in the recognition of these lesions, and do not accumulate SSB´s.

Alkylating agents like MMS and DMS give rise to methylated bases. Such lesions are removed by the conserted action of glycosylases and AP-endonucleases. This mechanism of **base excision repair** might result in short repair patches, a process for which xp cells are proficient. ApC caused accumulation of SSB both in normal and xp-cells previously exposed to MMS. The kinetics were similar as in UV-exposured normal cells.

A rapid increase in the level of SSB was seen both in normal and xp cells exposed to methylating agents upon the addition of inhibitors to poly(ADP-ribosyl)ation, like aminobenzamide (ABA). Simultaneous addition of ApC and ABA caused an additive increase in the number of SSB in these cells. ABA had no effect in cells exposed to UV.

Base and nucleotide excision repair were compared after exposure to one and the same agent by the use of a CHO cell line that expresses the den V gene, coding for a pyrimidine dimer specific glycosylase/AP-endonuclease. UV-exposed wild type and denV CHO cell lines by definition repair pyrimidine dimers by nucleotide and base excision, respectively. Both cell lines accumulated SSB with ApC present, but only the denV cells responded to ABA.

The data are interpreted in a model, where different parts of the genome are available to the two pathways of repair. The "UV-mimetic" endonuclease requires an "open" chromatin, while incision by glycosylase/AP-endonucleases during base excision may also take place in condensed chromatin. In this chromatin polymerases and ligases will have a limited access to nicked lesion-sites. A post incision modification of chromatin at these sites by poly(ADP-ribosyl)ation thus seems to give access to ligation. In a way, the two pathways of repair complement each other.

K 410 EXPRESSION OF THE HUMAN α-GLOBIN GENE IN RETROVIRALLY TRANSDUCED HAEMATOPOIETIC CELLS *N VIVO* Mitchell Finer and Richard Mulligan,Whitehead Institute for Biomedical Research, Cambridge MA
Many laboratories have demonstrated that retroviral vectors can be used to introduce foreign genes into bone marrow cells *in vivo* with high efficiency.Previous work has focused on the expression of cDNAs in direct orientation either from LTR based vectors or from a variety of internal promoters. Although high efficiency of gene transfer into bone marrow in long term reconstituted animals has been reported, these constructs express poorly or not at all *in vivo*. Instead, we have examined the expression of a 1.5 kb Pst I fragment encoding the complete human α1-globin gene and flanking sequences, cloned in reverse orientation into the retroviral vector pSG. pSG contains the 5' LTR and the first 1.4 kb of the gag region up to the Xho I site of Mo-MuLV joined by a Xho linker to the final 150 bp of Mo-MuLV from the Cla I site and the 3' LTR. The 3' LTR contained the Pvu II-Xba I enhancer deletion. This approach alleviates the artificial juxtapositioning of different promoter elements with cDNA coding sequences, during which sequences essential for mRNA stability, transport or correct translation might be improperly placed or deleted. Three ψ-2 producer clones have been isolated which transmit the correct viral structure at 20-30 copies per genome, assayed by 3T3 infection and southern blotting. These producers were used to infect mouse bone marrow in vitro followed by engraftment of lethally irradiated recipients The efficiency of short and long term engraftment with retrovirally marked stem cells was assayed by southern blotting. In addition, the expression of the transduced α-globin gene was assayed by SP6 mRNA protection. Seventy-five percent of the 14-day spleen colonies isolated were positive for single copy provirus integration at a unique site. Of these, 33% were positive for expression of human α-globin mRNA. The level of expression was position dependant. The highest levels of expression were comparable to that of the endogenous mouse γ-actin gene. Three months following transplant, DNA and RNA was isolated from blood, bone marrow, spleen and spleen B cells. Three animals were positive for integration in spleen and bone marrow DNA, the best animal containing a single copy of the provirus. SP6 mRNA protection revealed poor expression in blood, bone marrow and spleen B RNA, but high expression in total spleen RNA. This work demonstrates that the human α1-globin gene can be efficiently introduced into long term reconstituting cells. However, its expression is not specific to the erythroid lineage.

K 412 MODULATION OF THE PROCESSIVE NICKING ACTIVITY OF *Micrococcus luteus* U.V. ENDONUCLEASE,
Richard Hamilton and R. Steven Lloyd, Departments of Molecular Biology and Biochemistry, Center in Molecular Toxicology, Vanderbilt School of Medicine, Nashville, TN 37232
Micrococcus luteus U.V. endonuclease incises DNA at the sites of pyrimidine dimers. The mechanism of incision has been previously shown to be a glycosylic bond cleavage at the 5' pyrimidine of the dimer followed by an apyrimidinic endonuclease activity which cleaves the phosphodiester backbone between the pyrimidines. The process by which ***M. luteus*** U.V. endonuclease locates pyrimidine dimers within a population of U.V. irradiated plasmids was shown to occur, *in vitro*, by a processive or "sliding" mechanism on nontarget DNA as opposed to a distributive or "random hit " mechanism. Form I plasmid DNA containing 25 dimers per molecule was incubated with ***M. luteus*** U.V. endonuclease in time course reactions. The three topological forms of plasmid DNA generated were analyzed by agarose gel electrophoresis. When the enzyme encounters a pyrimidine dimer, it is significantly more likely to make only the glycosylase cleavage as opposed to making both the glycosylic and phosphodiester bond cleavages. Thus, plasmids are accumulated with many alkaline labile sites relative to single-strand breaks. In addition, reactions were performed at both pH (8.0) and pH (6.0), in the absence of NaCl as well as 25, 100, and 250 mM NaCl. The efficiency of the processive reaction was shown to be dependent on both the ionic strength and pH of the reaction. At low ionic strengths the reaction was shown to proceed by a processive mechanism and shifted to a distributive mechanism as the ionic strength of the reaction increased. Processivity at pH (8.0) is shown to be more sensitive to increases in ionic strength than reactions performed at pH (6.0). Supported by ES04091 and ES00267

K 413 INTRODUCTION OF GENES INTO RAT LIVER BY RETROVIRAL TRANSDUCTION: A POTENTIAL MODEL FOR HEPATIC GENE THERAPY,
Maria Hatzoglou, Anthony Wynshaw-Boris, Fatima Bosch, Frank Mularo and Richard W. Hanson, Department of Biochemistry and Pediatrics, Case Western Reserve University, Cleveland, OH 44106
Two methods are described in which chimeric marker genes were stabily introduced into the livers of rats by retroviral transduction. Retroviruses were injected into the peritoneal cavity of 14 to 22 day old rat fetuses in utero, or into the portal vein of adult rats following partial hepatectomy (removal of 70% of the liver). The marker genes were neo or bovine growth hormone (bGH), linked to the promoter/regulatory region of the rat gene for P-enolpyruvate carboxykinase (PEPCK). Provirus was integrated into DNA from the livers of infected animals for as long as 10 months after viral injection. Other tissues from these animals contained no provirus. Expression of the transduced genes was demonstrated by S1 nuclear mapping of RNA from the livers of infected animals. The presence of bGH protein was detected in the serum of animals infected with virus containing the PEPCK/bGH gene. The concentration of bGH in the serum of these animals was modulated by diet, in a manner consistent with the dietary regulation of the endogenous PEPCK gene in the livers of these animals. The specificity of the genetic modification, will be demonstrated by the histochemical localization of β-galactosidase to the livers of animals infected with virus containing this gene. These techniques provide a means to stabily modify gene expression in the liver of intact animals, and represents a potential model for the correction of liver specific genetic diseases. Supported in part by grants DK-21859 and DK-24451 from the N.I.H. and by funds from the Thomas Edison Program.

K 414 SUBCHROMOSOMAL MAPPING OF THE XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP-A LOCUS,
Karla Henning[1], Roger A. Schultz[2], Gurbax S. Sekhon[3] and Errol C. Friedberg[4], Departments of Genetics[1] and Pathology[4], Stanford University, Stanford, CA 94305, Division of Human Genetics, University of Maryland, Baltimore, MD 21201[2] and Division of Clinical Genetics, University of Wisconsin, Madison, WI 53705[3].
Previous studies resulted in the isolation of a monochromosomal human-mouse hybrid cell line designated K3, containing a single rearranged human chromsome which specifically complements the phenotype of xeroderma pigmentosum (XP) group A cells following microcell-mediated chromosome transfer. Cytogenetic and molecular characterization of this chromosome in A9 cells demonstrated that the q arm is from chromosome 11. However, the p arm of the rearranged chromosome consists of material from the region 9q34.1-9q34.3. Spontaneous deletion of the p arm of this chromosome was identifed in a derivative human-mouse hybrid designated 3B5A9. This deletion resulted in loss of detectable chromosome 9 sequences and loss of complementing ability in XP-A cells. The q arm of this chromosome appears to be unaltered. A second derivative hybrid cell line (212T1) was isolated that contains a complementing human chromosome. Southern hybridization analysis showed the loss of some chromosome 9 material. Based on these results we conclude that the XP-A locus is located near the distal end of the q arm of chromosome 9, between the c-*abl* gene located at 9q34.1 and a probe designated PS1.3, cytogenetically mapped by others to 9q34.3. Refined chromosomal mapping of the XP-A locus is expected to facilitate cloning the XP-A gene by screening genomic libraries of the 212T1 hybrid line with linked probes.

Biotechnology and Human Genetic Predisposition to Disease

K 415 MOUSE RETROVIRUS-LIKE (VL30) TRANSPOSABLE ELEMENT RNA IS ABUNDANTLY EXPRESSED IN PSI-2 HELPER CELLS, TRANSFERRED TO RECIPIENT RAT HEPATOMA CELLS, AND EXPRESSED IN A DEXAMETHASONE-INDUCIBLE FASHION ALONG WITH SPECIFICALLY VECTORED SEQUENCES: COMPLETE STRUCTURE. Clague P. Hodgson*, Renee Z. Fisk*, Maria Hatzoglu^, Paresh Arora+, Maqsood A. Chotani+, and Richard W. Hanson^, *Labs of Molecular and Developmental Biology/Dairy Science, The Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH. 44691; ^Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH 44106, + Department of Biology, College of Wooster, Wooster, OH. 44691.

Mouse VL30 sequences are a family of polymorphic transposable genetic elements found in mice. The RNA is co-packaged into murine leukemia virus virions, and replicates as defective retroviral RNA. Since psi-2 mouse cells and related lines are used for packaging defective retroviral vector RNA which will be used for human gene engineering trials, it is essential that they be demonstrably free of contaminating viral sequences which could co-infect, recombine with, and possibly replicate or cause neoplasms in the human recipient. Psi-2 cells were found to express large amounts of VL30 genomic RNA. Furthermore, rat hepatoma cells infected with psi-2 virions (carrying vectored neo gene sequences and selected on the drug G418) also bore expressible, dexamethasone-inducible VL30 sequences not found in pre-infection rat cells, demonstrating that they were transferred by psi-2 virions. The complete structure of a VL30 sequence of recent evolutionary origin from the balb C mouse genome is presented.

K 416 SPONTANEOUS MUTATION IN LYMPHOBLASTOID CELL LINES DERIVED FROM NORMAL AND BLOOM'S SYNDROME SUBJECTS. Caroline Ingle and Norman Drinkwater, McArdle Laboratory, University of Wisconsin, Madison, WI 53706 Bloom's syndrome is a rare autosomal recessive disorder showing a predisposition for the development of carcinomas and leukemias at an early age. Anomalies in DNA metabolism have been implicated in this syndrome but the basic defect has not been identified. By comparing the mutations arising spontaneously in cell lines derived from normal donors with those from patients with Bloom's syndrome we may gain some understanding of the basic defect and the origin of the predisposition for cancer. The genetic stability of the *oriP-tk* shuttle vectors derived from Epstein-Barr virus and the rate of accumulation of mutations in the HSV-*tk* target gene (1.9 mutation per 10^6 plasmids per cell generation) make it a valuable system for the study of spontaneous mutation. Using the *oriP-tk* shuttle vector, we are characterizing mutations (point mutations, deletions and insertions) that arise spontaneously and as a result of the process of transfection in lymphoblastoid cell lines of normal and Bloom's syndrome subjects. Preliminary results from transfection-induced mutants indicated the frequency of deletions (74%) in Bloom's syndrome cell lines is similar to that observed in normal cell lines (85%). In cloned normal cell lines where mutations arise from errors of replication, the frequency of deletions is 35%. Point mutations analysed so far include both transitions and transversions. Cloned lymphoblastoid cell lines from Bloom's syndrome subjects are currently being analysed for spontaneous mutations.

K 417 GERM-LINE CORRECTION OF THE OTC DEFICIENT *SPF* MOUSE, Stephen N. Jones, Marcus Grompe, Idrees Munir, and C. Thomas Caskey, Institute for Molecular Genetics, and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030
Ornithine transcarbamylase catalyzes the condensation of ornithine and carbamyl phosphate to form citrulline. The gene encoding OTC is located on the X chromosome in both man and mouse and is expressed primarily in liver and, to a lessor extent, in the mucosal layer of the small bowel. Research in OTC deficiency, one of the most common and severe inborn errors of ureagenesis in man, has been facilitated by the availability of an animal model for the disease - the sparse fur *(spf)* mutant mouse. The *spf* OTC gene contains a point mutation resulting in a gene product with an overall decrease in activity at physiologic pH and a change in pH optima from pH 7.7 to pH 9.5. The *spf* mouse offers an attractive opportunity to develop and test gene therapy strategies. As a first step toward gene therapy human OTC coding sequences were introduced into the germline of *spf* mice. Human OTC cDNA was placed under transcriptional control of 750 bp of mouse OTC 5' flanking sequences and linked to DNA sequences bearing the SV40 small t antigen splice sites and polyadenylation signal. Embryos were harvested from 7 superovulated *spf* homozygous females which had been mated with hemizygous *spf* males. 60 embryos were microinjected with approximately 500 copies of the transgene and transferred into 4 foster mothers. This experiment yielded 19 pups which survived the newborn period, 2 of which had a striking phenotype conversion to wt-like fur by day 9. Southern analysis of DNA isolated from tail cuts revealed that only the two mice with the phenotypic conversion bore the transgene. Several litters have been produced through matings of the transgenic mice with pure *spf* mice. The transgene is passed in a mendelian fashion and is linked with phenotype conversion in all cases. Tissues from these mice have been harvested and are currently being analysed for expression of the transgene at both the RNA and protein level.

Biotechnology and Human Genetic Predisposition to Disease

K 418 USE OF PCR TECHNOLOGY FOR THE DETECTION OF RETROVIRAL VECTORS AND REPLICATION COMPETENT AMPHOTROPIC RETROVIRUSES, Richard A. Morgan, Kenneth Cornetta, and W. French Anderson, Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892. Polymerase Chain Reaction (PCR) is a process whereby a specific region of DNA is amplified many thousand fold. We have developed procedures for the application of PCR to the detection of retroviral vectors and replication competent amphotropic retroviruses. These procedures are to be used in conjunction with our human gene transfer protocols. Primer pairs specific to the LTR, GAG, and ENV genes as well as a selectable marker gene (NEO) permit the rapid identification of transduced cells. Data will be presented on three specific applications of PCR technology in the study of retroviral vectors. (1) As a quick check for the integrity of the proviral structure, primer combinations in the LTR and internal sequences can identify deleted proviruses without Southern blotting. (2) The rapid estimation of vector titer can be accomplished on small amounts of viral supernant by first reverse transcribing isolated virion RNA and running the PCR assay in comparison to a virus stock of known titer. (3) The identification of small numbers of marked cells in biological samples can be accomplished and has been used to successfully follow the fate of transduced cells in vivo. PCR is also a sensitive means for the detection of replication competent helper virus in transduced cell populations, and can be used in the evaluation of the safety of gene therapy protocols.

K 419 THE RECOGNITION OF DAMAGED DNA IN YEAST AND HUMANS, Mark Patterson and Gilbert Chu, Department of Medicine, Stanford University Medical Center, Stanford, CA 94305. A gel eletrophoresis binding assay has been used to identify factors in humans which bind specifically to damaged DNA (Chu and Chang, Science 242:564, 1988). One strategy for the isolation of the genes which encode these factors is to study a simpler experimental organism such as the yeast, *Saccharomyces cerevisiae*. If analogous factors exist in yeast, it will be more straightforward to find the corresponding yeast genes; the yeast genes may then be used in turn to identify the homologous human genes.

We have found that yeast contains an activity which binds to damaged DNA. This activity appears to be similar to that identified in humans. First, the activity is localized in the nucleus. Second, the activity comprises two bands which differ in intensity in mobility shift gels. Third, UV damaged, cisplatin-damaged and undamaged DNA compete for the binding activity with the same relative efficiencies in yeast and humans. These results suggest that yeast and humans contain analogous activities which bind specifically to UV damaged DNA.

We are currently screening yeast mutant strains which are defective in the repair of DNA damage in order to identify the gene which encodes the binding activity.

K 420 INDUCIBLE SELECTIVE ABLATION OF HIV-tat EXPRESSING CELLS, Doros Platika, Elaine Dzierzak, and Richard C. Mulligan, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA 02142.

Human immunodeficiency virus (HIV) has been identified as the etiological agent in human acquired immunodeficiency syndrome (AIDS). Therapeutic strategies have concentrated on antiviral drugs such as AZT and on the development of a preventive vaccine. The molecular tools utilized in elucidating the structure and function of the virus have provided a third therapeutic strategy involving various molecular manipulations. Here we report a molecular strategy and preliminary results utilizing retroviral vectors for the specific ablation of HIV infected cells.

A number of recent studies have demonstrated the ablation of specific cell populations through the use of plasmids consisting of a tissue specific promoter driving a gene (such as diphtheria toxin) whose product is toxic to the cell. The promoter has to be highly cell specific since very few molecules of the toxic product are required for cell death. Borelli, et al. (1988) have described a system utilizing tissue specific promoters driving expression of the herpes simplex virus thymidine kinase gene (TK). TK is non-toxic in mammalian cells except in the presence of specific nucleoside analogues such as acyclovir. Toxicity can be varied by altering the level of TK expression, drug dosage and/or treatment duration. We have designed retroviral vectors containing a series of HIV promoter sequences driving the expression of TK. The vectors have been used to infect human cells. Northern analysis revealed the correct size TK transcript and that the presence of HIV transactivator protein (tat) increases TK expression for some of the vectors. Lastly, we have demonstrated that these cells can be selectively ablated with the use of acyclovir.

K 421 SELECTION AND DETECTION SYSTEMS FOR THE TARGETED MUTAGENESIS OF A HUMAN GENE OF UNKNOWN FUNCTION, *Andrew C.G.Porter, Biochemistry Department, Oxford University, Oxford OX1 3QU, UK.* The use of homologous recombination (HR) to modify, at will, the genes within living mammalian cells is desirable for the analysis of gene-function and the development of gene-therapy. Using as a model gene the human gene 6-16, whose function is unknown but whose transcriptional regulation by interferon (IFN) has been characterized, systems for the selection or detection of rare transfectants that have undergone HR have been designed. The transfected DNA contains a portion of the 6-16 gene as well as the selectable *gpt* gene and is incapable of expressing the latter unless HR between endogenous and exogenous 6-16 DNA occurs. Such recombination should silence the 6-16 gene and place the *gpt* gene under the transcriptional control of IFN so that the desired cells may be selected in HAT medium if IFN is present. Unwanted cells should die in HAT medium alone or in the presence of 6-thioguanine and IFN. Detection of the desired cells is based on the use of the polymerase chain reaction (PCR) to amplify a 1.1 kb sequence generated specifically by HR. The two systems have been tested on cells transfected with a plasmid that mimics the expected structure resulting from HR. Such cells show IFN-dependent *gpt* expression and growth in HAT medium and can be detected by PCR when their DNA is diluted at least 1000-fold with DNA from unmodified cells.

K 422 **ANTISENSE OLIGONUCLEOTIDE STRUCTURE: HYBRID FORMATION, NUCLEASE RESISTANCE, SENSITIVITY TO RNase H, AND EFFECT ON GENE REGULATION,** Robin S. Quartin[1], Joanne P. Spadoro[2], Christine L. Brakel[2] and James G. Wetmur[1], Department of Microbiology[1], Mount Sinai School of Medicine, New York NY 10029 and Enzo Biochem Inc.[2], 325 Hudson St., New York NY 10013. In order to determine the optimum structures for the design of antisense oligodeoxynucleotides, a series of oligodeoxynucleotides bearing different arrangements of nuclease-resistant internucleotide linkages was synthesized and examined both in vitro and in vivo. Optical melting temperature measurements of hybrids formed with complementary, all phosphodiester, sequences in high salt revealed that each internucleotide methylphosphonate incorporated produced a decrement in the free energy of hybrid formation of 0.75 kcal/mol. Similar results were obtained for carbamate internucleotide linkages. Nevertheless, Southern hybridization demonstrated that many uncharged linkages could be incorporated into an oligodeoxynucleotide without affecting specificity. Nuclease resistance of the oligodeoxynucleotides was detected, following digestion, by the ability to gel-shift a labeled complementary, all phosphodiester, oligodeoxynucleotide. Both 5' and 3' exonuclease function was blocked by two methylphosphonate linkages or by one linkage to a purine nucleoside. Methylphosphonate-containing oligodeoxynucleotides with 1-5 adjacent phosphodiester linkages were tested as substrates for the endonucleases DNase I and DNase II. The same methylphosphonate-containing oligodeoxynucleotides were hybridized to RNA runoff transcripts and tested as substrates for RNase H. The results indicate that the structural requirements for nuclease resistance are different from the requirements for formation of an RNase H substrate. Oligodeoxynucleotide sequences optimized for nuclease resistance and the ability to direct RNase H cleavage of complementary RNA were employed in an antisense assay for inhibition of production of a secreted gene product.

K 423 CLONING OF A DNA REPAIR GENE CONFERRING UV-RESISTANCE TO XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP A, A. Rinaldy, T. Bellew, E. Egli and R.S. Lloyd, Dept of Biochemistry and Center in Molecular Toxicology, Vanderbilt University Medical School, Nashville, Tennessee 37232
Accurate maintenance of a cell's genetic material requires mechanisms for repairing damage to DNA which is caused by a variety of chemical and physical agents. If DNA repair fails to occur, mutations may arise in the descendants which in turn can be phenotypically associated with neoplastic transformation (XP). A causal relationship between defective repair of UV damage to DNA and carcinogenesis is very strong in the disease of xeroderma pigmentosum. In order to understand the molecular basis of the initiation of normal DNA repair of pyrimidine dimers we have cloned and characterized a gene which confers enhanced UV survival of an XP-A cell line. This was done by using a 10.6S size cut cDNA libraries of the heterozygous non affected mother's cell line and homozygous affected child's cell line by a competition hybridization technique. A fragment of the cDNA clone was used to screen a human cosmid library (108HL) which had been constructed using the expression vector pCV108 containing a neomycin-resistance gene marker. The XP-A cell line was individually tranformed with six genomic clones and colonies were selected for neomycin-resistance and then UV- resistance. The results indicated that one cosmid could confer not only neomycin resistance but also a significant enhancement in UV survival of the transformants as assayed by post-UV colony forming ability. Slot blot analyses of RNA's extracted from these UV-resistant clones and the non-transformed XP-A cell line demonstrated that there is a good correlation between UV survival and expression of this 10.6S mRNA. These data indicate that the lower level of expression of this gene may be responsible for the disease state of xeroderma pigmentosum. Supported by CA43769.

K 424 ANALYSIS OF RETROVIRAL TRANSCRIPTION AND PROVIRAL METHYLATION IN HEMATOPOIETIC CELLS IN LONG-TERM RECONSTITUTED ANIMALS, **Paul D. Robbins, Pierre Lehn, and Richard C. Mulligan, Whitehead Institute, Cambridge, MA 02142.** Expression from the Moloney virus LTR is extremely low in undifferentiated EC cells and in hematopoietic cells in *vivo*. To overcome the apparent block to retroviral transcription we have replaced the Moloney enhancer with the SV40, Polyoma F9.1, and MPSV enhancer elements. We have introduced the defective retroviral vectors containing the heterologous enhancer elements into a helper-free packaging line (CRE) to generate high titer producers. The producers were used to infect either EC cells or 5-FU treated bone marrow that was then transplanted into lethally irradiated mouse recipients. The presence of each of the heterologous enhancer elements signifiancdy increased retroviral transcription in EC cells and in *vivo* in long-term reconstituted animals. We have used retroviral constructs containing the MPSV enhancer to express the cDNA encoding DHFR* in long-term reconstituted animals. Expression of DHFR* should allow for the selection of infected stem cells in vivo by injection of the appropriate dose of methotrexate. Furthermore, we have shown that the proviral DNA in EC cells becomes hypermethylated 7-14 days post-infection, independent of the enhancer present. In contrast, the proviral DNA in long term-reconstituted primary and secondary animals is only partially methylated and the extent of methylation is dependent upon the site of viral integration. Furthermore, the proviral methylation pattern in different lineages derived from the same animal is identical which suggests that the partial proviral methylation occurs in early progenitor cells. It is not clear, however, whether the partial methylation reduces retroviral transcription in *vivo*. Using the retroviral integration sites as markers, we have also observed that multiple stem cells can contribute to the reconstitution of transplanted animals. Furthermore, stem cells identical to the reconstituting cells in a primary animal can reconstitute multiple secondary animals. We have also observed that stem cells not detected in a primary animal can contribute to the reconstitution of secondary animals. These results suggest that stem cell renewal and repopulation is a dynamic and variable process.

K 425 **ANTISENSE HPRT RNA PRODUCTION IN MAMMALIAN CELLS AND TRANSGENIC MICE**

Belinda J.F. Rossiter, M. Idrees Munir and C. Thomas Caskey
Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

One potential method for generating mouse models for human diseases is the antisense inhibition of specific genes *in vivo*. We have demonstrated a reduction of hypoxanthine guanine phosphoribosyltransferase (HPRT) activity to undetectable levels in mouse fibroblasts following expression of antisense HPRT RNA in these cells. Given this success in cultured cells, we have generated two series of transgenic mice which express antisense HPRT RNA in different tissues. We have examined the effect of this antisense HPRT expression on endogenous HPRT message levels, and on the amount and activity of the HPRT enzyme. Some reduction in enzyme activity has been observed.

K 426 HIGH LEVEL EXPRESSION OF A RETROVIRALLY TRANSFERRED GENE IN A TRANSGENIC MOUSE. Peter B. Rowe, Karen Brennan, Eva Fiala-Beer and Stephen A. Wood. Children's Medical Research Foundation, Camperdown, N.S.W. Australia, 2050.
It has been difficult to achieve significant levels of expression of retrovirally transferred genes in either cultured cells or transgenic animals. This has been variously ascribed to low titres of the replication-defective viruses, DNA methylation in embryonic cells associated with retroviral provirus integration, low levels of LTR promoter function, suppression of internal promoters and the absence of introns from the gene cDNA's that are employed for reasons of convenience and size limitation. We have created a transgenic mouse by infecting a 4-cell stage embryo with a defective retrovirus (MoMuLV) containing a neomycin resistance gene under the control of the 5'-LTR and a v-myc (MC29) gene under the control of an M.H.C. Class I ($H2K^b$) promoter. This mouse which could not be bred was sacrificed at 10 weeks of age and exhibited splenomegaly, thymus enlargement and generalised lymphadenopathy. The normal structure of these organs was totally replaced by lymphoblastic cells and the peripheral blood contained large numbers of primitive haemopoietic cells. Northern analysis confirmed high levels of expression of v-myc in most tissues as might be expected from the generalized expression of the $H2K^b$ gene in post natal tissues. It would appear that the malignant changes in the haemopoietic cells resulted from a second mutational event.

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K 427 EPIGENETIC EFFECTS OF SV40 ON DNA REPAIR, N.V.Somia, D.W.Melton, Dept. of Molecular Biology, University of Edinburgh EH9 3JR UK.
Xeroderma Pigmentosum, Cockaynes syndrome and Blooms syndrome are autosomal recessive diseases with DNA processing defects. Molecular studies of DNA repair in human cells have been hampered by the limited ability of mutant cells to integrate exogenous DNA stably. This problem has been partly overcome by using mutant cells of other species and complementing them with human DNA.
One such mutant, an SV40 transformed Indian Muntjac cell line (SVM) exhibits an array of defects which resemble the above mentioned diseased cells. For example, SVM is defective in 'post replication repair' (PRR), i.e. the ability to use damaged DNA as a template for synthesis. In this it resembles the Xeroderma Pigmentosum variant cell. Using mouse DNA partial complementation of the PRR defect has been achieved (after UV selection), but there exists a possibility that the SV40 component may exert epigenetic effects which partly mimic a rescued phenotype. To clarify this we have looked at aspects of SV40 in the cell.
We have isolated spontaneous UV^R revertants and defined changes in their SV40 components. These changes are probably significant given the hypomutability of the cell line. The T-antigen encoded by SV40, known to affect several features of cellular metabolism, is present as a super T-antigen (100 Kd) and a truncated T-antigen (76 Kd), and so departs from the wild type form (90 Kd). However, using five antibodies spanning the protein, we have been unable to define any quantitative or qualitative changes between the mutant and revertants.
A change also exists, at the DNA level, in the SV40 integration pattern of the revertants. This change results in a duplication of the enhancer region of SV40 bordering the virus-cell junction. We are presently examining the sequences flanking the SV40 integration site to test the possibility that SV40 has acted as an insertional mutagen into sequences required for DNA repair.

K 428 DOUBLE STRAND CLEAVAGE OF LARGE DNA AT A SINGLE SITE BY TRIPLE HELIX FORMATION, Scott A. Strobel, Heinz E. Moser, and Peter B. Dervan, Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology, Pasadena, CA 91125. An oligonucleotide-EDTA-Fe probe (0.8 μM) equipped with thymine-EDTA at the 5' end, 5'-T*$T_3CT_6CT_4CT$-3', causes double strand cleavage at a single homopurine site 18 base pairs in size (5'-$A_4GA_6GA_4GA$-3') within 48,502 base pairs of the bacteriophage λ genome (4 μM base pairs.) The double strand cleavage efficiency is 25% (100 mM NaCl, 25 mM tris acetate, pH 7.0, 1 mM spermine, 24 °C). No secondary cleavage sites at partially homologous sequences were detected under these reaction conditions. The oligonucleotide-EDTA-Fe mediated site specific double strand cleavage can be carried out in low melting point agarose with comparable efficiency. This work has implications for isolating genes from large chromosomes and mapping large genomes.

K 429 TARGETING OF FRAMESHIFT MISMATCH CORRECTION DURING EXCISION REPAIR OF DNA URACIL, Nikolai V. Tomilin, Vita M. Golubovskaya and Olga N. Aprelikova, Institute of Cytology, The Academy of Sciences of the USSR, 194064 Leningrad, USSR. Single-stranded DNA of the phage M13 grown in *E. coli* <u>dut</u>⁻ <u>ung</u>⁻ double mutant (deficient in dUTPase and uracil-DNA glycosylase) contains 20-30 uracil residues. After introduction of the phage into E.coli <u>ung</u>+ (but not <u>ung</u>⁻) cells its DNA rapidly degrades and the effect is supressed by <u>ung</u>⁻ mutation. In vitro synthesis of the complementary DNA strand in the absence of dUTP using oligonucleotide primer and phage T4 DNA polymerase (even in the absence of DNA ligase) provides the template for UDG (uracil-DNA glycosylase)-driven excision repair in vivo. When the in vitro synthesis is efficient, the survival of half-uracil-substituted DNA in <u>ung</u>+ host is about the same as in <u>ung</u>⁻cells. We have used in this system the oligonucleotide primer containing frameshift mutation (-1) in the polylinker region of M13mp18, and measured the number of oligonucleotide-induced <u>lac</u>⁻ mutations after efficient in vitro synthesis on uracil-substituted template. We have found that the absolute number (as well as the frequency) of <u>lac</u>⁻ mutations is about 5 times higher when DNA is transfected into <u>ung</u>+ cells as compared to <u>ung</u>- cells. This result cannot be interpreted via selective degradation of uracil-containing strand, and we suggest that UDG-driven repair directs the mismatch correction toward uracil strand.

K 430 PRODUCTION OF HBsAg IN MOUSE FIBROBLASTS/BPV-VECTOR EXPRESSION SYS*

PRODUCTION OF HBsAg IN MOUSE FIBROBLASTS/BPV-VECTOR EXPRESSION SYSTEM USING HUMAN METALLOTHIONEIN PROMOTER,
Yan-bo Xie, Rong-guo Qiu, Ling-rong Liang, Molecular Biology Research Laboratory, Sun Yat-sen University of Medical Sciences, Guang-zhou, China.

A transcriptional cassette consisted of human metallothionein gene promoter II (HuMT-II), HBsAg gene and SV40 early gene transcriptional processing signals has been constructed. The cassette was inserted into a modified BPV-plasmid to form plasmid pdMTsAg-5. Mouse fibroblasts C127 were transfected with this plasmid and the transformants could produce comparable amount of HBsAg. The expression of HBsAg in the transformed cell line MT-5 is genetically stable and is inducible by Zn^{2-} and Cd^{2-} but not by dexamethasone. The antigen is released into the culture supernatant as 22-nm particles, having the same buoyant density as those derived from human serum. SDS-PAGE analysis showed two characteristic polypeptides of 23K and 27K in the HBsAg expressed by MT-5 cells. The highest yield of HBsAg in this cell line reached 2.5 mg/l, that is , 2.2×10^{-12}g per cell. The tranformed cells retain the pdMTsAg-5 plasmid in extrachromosomal state with a copy number of about 10 to 30 per cell. There is a rough correlation between copy number and the expression level of HBsAg.