MOLECULAR BIOLOGY OF THE EYE: GENES, VISION & OCULAR DISEASE

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Molecular Biology of the Eye: Genes, Vision & Ocular Disease

Keynote Address

I 001 RETROVIRAL VECTOR - MEDIATED GENE TRANSFER - AN APPROACH TO GENETIC CORRECTION OF HERITABLE DISEASE, C. Thomas Caskey, Grant R. MacGregor, Frederick A. Fletcher, Kateri A. Moore and John W. Belmont, Howard Hughes Medical Institute, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

The treatment of recessively inherited diseases of the eye such as galactosemia, cystinosis, and gyrate atrophy presently relies upon dietary management to reduce toxic metabolites. The somatic mutation of the rb gene, which accounts for retinoblastoma, is presently managed by surgery and chemotherapy. Techniques are now available to deliver and express foreign genes in cells in culture, primary hematopoietic cells, and, as described below, long term reconstituted mice. Gene replacement therapy may have a role in selected heritable and acquired eye diseases. We have focused our research efforts on the development of such methods using ADA deficiency resultant combined immunodeficiency as a model.

Sixteen replication-defective retrovirus vectors which carry human adenosine deaminase (ADA) coding sequences were constructed to evaluate several functional parameters: (a) extended viral *gag* gene sequences in packaging of defective particles; (b) internal promoters in independent transcriptional units; and (c) altered 3' LTR's in packaging and expression. Each vector was transfected into the amphotropic packaging cell line PA317 or the ecotropic packaging cell line ψ_2 and stable transformant populations were tested for their ability to transduce ADA to Rat 208F target cells. Virus containing supernatants from populations which were positive in the initial screen were used to infect the ecotropic packaging cell line ψ_2. Individual ψ_2 clones were then tested for ADA transduction. These studies indicated that optimal production of virus was dependent on the region of *gag* 3' to the classical ψ packaging sequence. Vectors with altered 3' LTR's function poorly in the production of virus.

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

Phototransduction - I

I 002 RHODOPSIN: STRUCTURE, FUNCTION AND TOPOGRAPHY. Paul A. Hargrave, University of Florida College of Medicine, Gainesville, FL 32610.

Rhodopsin is the photoreceptor protein of rod cells of the retina. We know most about the properties of bovine rhodopsin, although the sequences of as many as a dozen different visual pigments have been determined (including those from human and Drosophila).

Rhodopsin is a single-chain integral membrane protein located in disk membranes in the outer segment of rod cells. In most verterbrates the rod cell visual pigment is 348 amino acids in length. About 50% of its mass is embedded in the lipid bilayer; 25% is exposed to the intradiskal space and 25% to the cytoplasm. It is thought to be composed of a bundle of 7 transmembrane helices which form a binding pocket for it's chromophore, retinal. The 11-cis isomer of retinal is attached to a specific lysine, Lys^{296}, which is located midway in the 7th transmembrane segment. Upon absorption of a photon of light, the 11-cis retinal isomerises to all-trans. The protein subsequently undergoes a change in conformation which serves as the mechanism by which light-dependent biochemical changes are initiated. A G-protein, termed transducin, binds to the illuminated form of rhodopsin (or, opsin). Following a GDP-GTP exchange, transducin activates cGMP phosphodiesterase. The subsequent drop in intracellular cGMP causes a shut-off of ion channels in the plasma membrane, causing membrane hyperpolarization which leads to communication with other cells in the retina.

Following light reception, opsin kinase phosphorylates up to 9 serine and threonine residues on opsin's cytoplasmic surface. This reduces the ability of opsin to further activate transducin. A 48K protein selectively binds to the surface of phosphorylated opsin and further "deactivates" opsin. Phosphorylation sites are located in a serine and threonine-rich region of the carboxyl-terminus and in the cytoplasmic loop connecting helices V and VI. Limited proteolysis experiments suggest that this cytoplasmic loop V-VI is also involved in interaction with the G-protein, transducin. Other regions such as loop I-II are well conserved phylogenetically, indicating that they are involved in conserved functions. Sequence comparison of different species of rhodopsin show that two cysteines are invariant, suggesting that they are involved structurally in a disulfide bond.

Comparison of rhodopsin sequences, and probe hybridization experiments, support the hypothesis that photosensory pigments may have evolved from a common precursor. Comparison of sequences of rhodopsins with those of other receptors suggest that rhodopsin, β-adrenergic receptor and the muscarinic acetylcholine receptor belong to the same superfamily of receptors which will contain many other members which operate through G-proteins.

I 003 FUNCTIONAL ANALYSIS OF RHODOPSIN MUTANTS EXPRESSED IN MONKEY KIDNEY CELLS. Thomas P. Sakmar, Sadashiva S. Karnik, Roland R. Franke, Barry E. Knox, Daniel D. Oprian, Hai-Bao Chen, and H. Gobind Khorana, Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

We have expressed a synthetic gene for bovine rhodopsin (1) in monkey kidney cells (COS-1) to the level of 0.3% of cell protein. After in vivo reconstitution with 11-cis retinal, the COS-1 cell rhodopsin was purified by immunoaffinity adsorption. The purified COS-1 cell rhodopsin had a visible absorption spectrum indistinguishable from that of retinal rhodopsin. Further, the COS-1 cell rhodopsin enhanced the GTPase activity of transducin in a light-dependent manner with the same specific activity as the native retinal pigment. We have turned our attention to structure-function studies on rhodopsin by site-specific mutagenesis. Three mutants were constructed in which charged amino acid residues in the cytoplasmic loop linking putative transmembrane helices E and F were replaced by neutral residues: mutant 1; Glu239→Gln, Lys248→Leu, Glu249→Gln. The three mutants were expressed in COS-1 cells and purified. Whereas mutants 1 and 3 activated transducin normally, mutant 2 did not activate transducin in our assay. Mutagenesis studies have also been undertaken to investigate the role of cysteine residues in bovine rhodopsin. Current models of rhodopsin structure with respect to the disk membrane bilayer (2) distribute the 10 cysteine residues as follows: 3 cysteines are on the intradiskal surface, 4 cysteines are embedded within the bilayer, and 3 cysteines are located on the cytoplasmic carboxyl terminal tail. Using this model as a basis for design, 7 mutants were constructed in which cysteine residues were replaced by serine residues. The mutant genes were expressed in COS-1 cells. Characterization of the 7 rhodopsin cysteine mutants is underway. To elucidate the role of rhodopsin phosphorylation in visual transduction, 5 mutants in which carboxyl terminal domain serine or threonine residues are replaced by alanine have been constructed. Characterization of these phosphorylation mutants is also underway. Supported by grants from the O.N.R. and N.I.H. Thomas P. Sakmar is supported by a fellowship from N.I.H.; Roland R. Franke is supported by a scholarship from the German Academic Exchange Service and Barry E. Knox is supported by a fellowship from N.I.H.

(1) Ferretti, L., Karnik, S. S., Khorana, H. G., Nassal, M., and Oprian, D. D. (1986) Proc. Natl. Acad. Sci. USA 83, 599-603.

(2) Dratz, E. A. and Hargrave, P. A. (1983) Trends Biochem. Sci. 8, 128-131.

I 004 PROTEINS INVOLVED IN THE ACTIVATION AND DEACTIVATION OF cGMP-PHOSPHODIESTERASE IN RODS. Hermann Kühn and Ursula Wilden, Institut für Biologische Informationsverarbeitung der Kernforschungsanlage Jülich, 5170 Jülich, FRG

Photoactivated rhodopsin (R^*) specifically interacts with the following three proteins in rod outer segments.

(i) A guanine nucleotide binding protein (G-protein, "transducin") binds to R^* in a 1 : 1 complex; this enables exchange of GTP for previously bound GDP on the α-subunit (T_α) of transducin. After nucleotide exchange, the protein complex (R^*- $T_{\alpha\beta\gamma}$) dissociates into R^*, $T_{\beta\gamma}$, and T_α-GTP which then activates cGMP-phosphodiesterase. A single R^* catalyzes GDP/GTP exchange on hundreds of transducin molecules, thereby providing the first step of amplification of the photon signal.

(ii) A protein kinase ("rhodopsin kinase"), which is independent of cyclic nucleotides or Ca^{2+}, binds and phosphorylates photobleached but not unbleached rhodopsin at multiple (maximally nine) serine and threonine sites.

(iii) A soluble protein called "48 K-protein" (or "S-antigen" or "arrestin") also binds to R^*, in particular to phosphorylated R^* ($P\text{-}R^*$).

Phosphorylation lowers R^*'s affinity for transducin, and strongly increases R^*'s affinity for arrestin. Phosphorylated R^* has a reduced catalytic capacity to activate transducin and PDE, even in the absence of arrestin. Binding of arrestin to $P\text{-}R^*$ further decreases the capacity of $P\text{-}R^*$ to activate the PDE enzyme cascade (1). The role of different individual phosphorylation levels of rhodopsin will be discussed.

An analogous system is the β-adrenergic receptor whose deactivation also requires receptor phosphorylation and the binding of an arrestin-like protein (2).

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Phototransduction - II

I 005 TRANSDUCINS, Connie L. Lerea*, Carol Raport*, Beverley Dere*, Ann Hobson*, Ann Bunt-Milian# and James B. Hurley*, *Dept. of Biochemistry and Howard Hughes Medical Institute and #Dept. of Ophthalmology, University of Washington, Seattle, WA 98195.

Vertebrate rod and cone photoreceptors each express a unique form of the photoreceptor specific G-protein, transducin. Two different cDNA clones that correspond to rod and cone transducin α subunits were isolated and characterized from a bovine retina cDNA library. The polypeptides encoded by these cDNA clones were localized to rod and cone cells by immunocytochemical analyses of sections of bovine retinas. Antibodies generated against synthetic polypeptides having sequences derived from the two cDNA clones were used in these experiments. The bovine cDNA clones were also used to identify and isolate corresponding human cDNA clones. Antibodies raised against synthetic peptides with sequences derived from the human transducin α subunits react specifically with human rod and cone photoreceptors in sections of human retina. In order to determine whether red and green cones express the same form of transducins as blue cones, it was necessary to develop probes that specifically identify red, green and blue cones. Antibodies that recognize red and green opsins and ones that recognize blue opsin were generated against synthetic peptides with sequences derived from the red, green and blue opsin amino acid sequences. Data obtained from double label analyses of human retina sections using anti-opsin antibodies and anti-cone transducin antibodies suggest that the red, green and blue cones either express the same cone transducin α subunit gene or different genes that encode nearly identical proteins. Antibodies that recognize two different regions of the transducin molecule were used in these experiments.

Two polypeptides that react with the cone transducin α subunit anti-peptide antibody have been purified from bovine retinas and they are being characterized to determine whether or not they have transducin like activities. We plan to determine whether or not kinetic differences between rod and cone transducins can partially account for physiological differences between rods and cones. Finally, the mouse rod transducin α subunit has been cloned and characterized. There are five introns within this gene and three of them are found in identical positions in the mouse transducin gene and in a Drosophila G-protein α subunit gene. A likely evolutionary history of G-protein subunits has been determined based on estimates of the number of third base substitutions that have occurred in corresponding codons between pairs of G-protein subunit sequences.

I 006 ISOLATION AND CHARACTERIZATION OF THE GENE ASSOCIATED WITH EYE-SPECIFIC PHOSPHOLIPASE C ACTIVITY, Brian Bloomquist[1], Randall Shortridge[1], Craig Montell[2], Hermann Steller[2], Gerald Rubin[2], and William L. Pak[1], [1]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907; [2]Department of Biochemistry, University of California, Berkeley, CA 94720.

Several lines of evidence suggest that the norpA gene is integrally involved in the phototransduction process in Drosophila. Severe norpA mutations render flies blind by eliminating the physiological responses of the photoreceptor. Earlier work has indicated that the norpA gene encodes a protein directly involved in an intermediate step of phototransduction. More recently, Inoue et al. (1985) have reported that norpA mutations affect the phospholipase C activity in the eye. The distal breakpoints of two gamma-ray induced deficiencies, Df(1)rb41 and Df(1)biD2, define the location of norpA to the interband region 4B6-4C1. The distal breakpoints of these deficiencies are cytologically indistinguishable; yet, rb41 deletes the norpA function, while biD2 leaves it intact. H. Steller and C. Montell of G. Rubin's laboratory have isolated two cosmids containing DNA from the 4BC region. By in situ hybridization to polytene chromosomes, we have determined that all of the 50 kb insert DNA lies within the norpA region as defined by the deficiency mapping. We looked for regions within the insert DNA that are transcribed only in the head. While there are several regions for which transcripts are found in the adult fly, only one region was found to be head-specific. We have isolated a norpA allele in P-M dysgenesis experiments. Upon further analysis, the mutant was shown to contain a hobo element within the head-specific region. Forty cDNA clones have been isolated from a screen of seven million pfu using genomic DNA from the head-specific region as a probe. The results of the analysis of the cDNA clones and of the transcripts encoded by the head-specific region will be discussed.

I 007 COUPLING OF PHOTOEXCITED RHODOPSIN TO INOSITOL PHOSPHOLIPID HYDROLYSIS IN FLY PHOTORECEPTORS.

Z. Selinger and B. Minke, Departments of Biological Chemistry and Physiology. The Hebrew University of Jerusalem. Jerusalem, 91904 Israel.

ABSTRACT Fly photoreceptor membranes were used to test the effect of light and of compounds causing photoreceptor excitation on defined biochemical reactions. Complementary electrophysiological studies examined whether putative second messengers excite the fly photoreceptor cells. This analysis revealed the following sequence of events: photoexcited rhodopsin activates a G protein by facilitating GTP binding. The G protein then activates a phospholipase C that generates inositol trisphosphate, which in turn acts as an internal messenger to bring about depolarization of the photoreceptor cell. Binding assays of GTP analogs and measurements of GTPase activity showed that there are 1.6 million copies of G protein per photoreceptor cell. The GTP binding component is a 41-kDa protein, and the light-activated GTPase is dependent on photoconversion of rhodopsin to metarhodopsin. Analysis of phospholipase C activity revealed that this enzyme is under stringent control of the G protein, that the major product formed is inositol trisphosphate, and that this product is rapidly hydrolyzed by a specific phosphomonoesterase. Introduction of inositol trisphosphate to the intact photoreceptor cell mimics the effect of light, and biphosphoglycerate, which inhibits inositol trisphosphate hydrolysis, enhances the effects of inositol trisphosphate and of dim light. Furthermore studies of temperature sensitive mutant with no receptor potential at the restrictive temperature showed that hydrolysis of phosphoinositide was abolished at this temperature while light dependent GTPase and diglyceride kinase activities were undiminished. The interaction of photoexcited rhodopsin with a G protein is thus similar in both vertebrate and invertebrate photoreceptors. These G proteins, however, activate different photoreceptor enzymes; phospholipase C in invertebrates and cGMP phosphodiesterase in vertebrates.

I 008 STRUCTURAL AND FUNCTIONAL ELUCIDATION OF S-ANTIGEN, Toshimichi Shinohara, Kunihiko Yamaki, Masahiko Tsuda, [1]Larry A. Donoso and Benjamin Amaladoss, Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD 20892, [1]Wills Eye Hospital, Philadelphia, PA 19107.

S-Antigen is a soluble protein present in retina and pineal gland having a molecular weight of 4.5 kilo daltons. This protein is considered to modulate amplified phototransduction cascade by binding to photoactivated phosphorylated rhodopsin (R*P). There are large amounts of S-Ag but no or minute amount of rhodopsin in the pineal gland suggesting that S-Ag must have a function independent of rhodopsin. Also, this protein is highly antigenic and responsible for the induction of experimetnal autoimmune uveitis (EAU).

In order to understand its functional role, we have determined human, bovine and mouse S-Ag polypeptide sequences and cDNA sequences. All of them had similar sequences but distinct differences were observed at multiple sites both in the protein and DNA. No extensive sequence homology was found between S-Ag and other proteins. However, local regions of sequence similarity with α-transducin are apparent including the pertussis toxin ADP-ribosylation site and phosphoryl binding sites. Although we proposed a binding model between S-Ag and rhodopsin, there is no crucial evidence to prove that S-Ag competitively binds to R* at the same site as α-transducin. S-Ag binds more firmly to R*P because there is not only a rhodopsin binding site but also a phosphate binding pocket. In order to probe further we have synthesized oligopeptides and competition binding experiments were performed between S-Ag and oligopeptides to the rhodopsin. Also we used S-Ag monoclonal antibodies to block a binding between S-Ag and rhodopsin. These results will be discussed.

We have tested for rhodopsin kinase and rhodopsin phosphatase activity in the highly purified S-Antigen which was detected. Southern Blot hybridization analysis clealy indicates that S-Antigen is a single copy gene in the mouse and it must be expressed from the same gene in retina and pineal gland.

Phototransduction - III

1009 LOCALIZATION OF α SUBUNITS OF SEVERAL SIGNAL TRANSDUCING G-PROTEINS AND THEIR CORRESPONDING mRNAs WITHIN RETINA, BRAIN AND AMONG PERIPERAL TISSUES AND CELL LINES, Mark R. Brann and Allen Spiegel, Metabolic Diseases Branch, NIDDK, Bethesda MD 20892. Signal transducing G-proteins consist of α β and γ subunits, the α subunits are apparently unique for each functionally defined protein. The sequences of the α subunits of these G-proteins have recently been deduced from cDNAs: transducin which mediates activation of retinal cGMP phosphodiesterase; Gs, which mediates stimulation of adenylate cyclase; Go, a major pertussis toxin substrate of brain; Gi1, Gi2, and Gi3, three structurally homologous proteins related (identical) to the G-protein(s) which mediate inhibition of adenylate cyclase. To localize mRNAs encoding these G-proteins we prepared a series of synthetic oligodeoxynucleotide cDNA probes for use in Northern blots and *in situ* hybridizations. To localize the encoded proteins we prepared a series of synthetic peptides, based on the deduced protein sequences, and prepared polyclonal antibodies. Within the retina, transducin was observed only in photoreceptors. Within photoreceptors, the mRNA was located primarily in the inner segments, while the location of the protein depended on the light-dark cycle, being most abundant in the inner segments during the day and outer segments at night. Go was not present in photoreceptors, but was found in the plexiform layers of the neural retina. Gs and Gi2 mRNA were observed to be most abundant within the brain, but were also observed in all tissues and cell lines examined. Within the brain, these two mRNAs showed similar regional distributions. Go and Gi1 mRNA were only observed in the brain, retina and in neuronal cell lines. Within the brain, the distributions of Go and Gi1 mRNAs were distinct from each other and from Gi2 and Gs mRNA. Gi3 mRNA was barely detectable in the brain, but was abundant within liver and kidney and in all cell lines examined. Data from western blots with the antibodies paralleled the mRNA data concerning the distribution of the proteins among tissues and cell lines. On the other hand, the relative concentrations of the mRNAs did not predict the relative concentrations of the encoded proteins within the individual tissues and cell lines. For example, in brain the relative concentrations of the mRNAs were Gs>Gi2>Go>Gi1>Gi3 and for the proteins they were Go>Gi1>Gi2>Gs. These data illustrate that the G-proteins are tissue specific in their expression, and the mRNA concentrations do not predict the relative concentrations of the encoded proteins.

1010 THE CELLULAR RETINALDEHYDE-BINDING PROTEIN

John W. Crabb, Steven Goldflam, Charles M. Johnson, Steven A. Carr, Steven E. Harris and John C. Saari, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY 12946, SmithKline and French Laboratories, Swedeland, PA 19479 and University of Washington, Seattle, WA 98195. Cellular retinaldehyde-binding protein (CRALBP) is likely to be a functional component of the visual cycle, perhaps serving as a stereoselective agent and/or substrate carrier protein. Distinct from other retinoid-binding proteins, CRALBP has only been found in retina, retinal pigment epithelium (RPE), and pineal organ and carries 11-cis-retinaldehyde and/or 11-cis-retinol as endogenous ligands. 11-cis-retinaldehyde bound to CRALBP is unaffected in bovine eyecup preparations by illumination that bleaches ~70% of the rhodopsin. The low photosensitivity of the CRALBP·11-cis-retinaldehyde complex suggests a protective role for the protein. The ability to select 11-cis-retinaldehyde from a mixture of geometrical isomers suggests that CRALBP could function as a component of the mechanism for the generation of 11-cis-retinaldehyde in the dark. An 11-cis-retinol dehydrogenase from RPE microsomes reduces 11-cis-retinaldehyde complexed with CRALBP and the reaction product, 11-cis-retinol, remains protein-bound. In contrast, a retinyl ester synthase also in RPE microsomes will readily esterify 11-cis-retinol bound to CRALBP, resulting in the dissociation of the retinoid from CRALBP. Perhaps CRALBP, the 11-cis-retinol dehydrogenase and the retinyl ester synthase interact to direct the metabolic flux of retinaldehyde in the RPE. As part of our continuing study of the role of CRALBP in the physiology of the retina we have directly characterized the protein purified from bovine retina as well as cloned and sequenced the cDNA encoding the CRALBP from both bovine and human retinal cDNA libraries. Bovine and human CRALBP are 91% identical and not related to any other proteins in the National Biomedical Research Foundation Sequence data base. Present efforts are directed toward cloning and characterizing the human gene for CRALBP, in situ hybridization and chromosomal localization. (Supported in part by USPH Grants, EY-02317, EY-06603 and CA-37589 and by RJR Nabisco).

I 011 MOLECULAR GENETICS OF INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN. J.M. Nickerson, D. Borst, T.M. Redmond, J-S. Si, B. Hershfield, A. Albini, L. Inouye, J. Toffenetti and G.J. Chader, NEI, NIH, Bethesda, MD 20892

Interphotoreceptor retinoid-binding protein (IRBP) is a large lipoglycoprotein (MW 146,000 in cattle) found in the subretinal space of man and other vertebrates. IRBP is thought to play a key role in the transport of retinol between the pigment epithelium and the photoreceptors. We have isolated several bovine cDNA clones and shown that they encode bovine IRBP. The composite cDNA sequence of these clones is 3.7 kb long and contains a poly(A) tail. The IRBP mRNA is long (7000 bases) and gives only one band on a Northern blot; yet there may be sequence heterogeneity near the 3' end of the IRBP mRNA. Two cDNA clones (one from our group and one from Bridges and co-workers) show a striking divergence in their sequences, yet sequences both 5' and 3' to the divergence are identical. The cDNA sequences have been used to predict the amino acid sequence of the protein. These sequences have been helpful in the analysis of the uveitogenic peptides in IRBP. The entire sequence of a uveitogenic CNBr fragment has been obtained from the cDNA sequence, allowing the search for the smallest uveitogenic agent to be further concentrated to within this fragment. Synthesized oligopeptides are now being used to elicit a uveitogenic response. The entire gene for bovine IRBP has been cloned. About 11 kb of 17 kb of the complete gene clone have been sequenced. The authentic N-terminus, the putative initiator methionine codon and a putative signal peptide sequence of the IRBP polypeptide have been identified from the gene sequence. At least five exons and four introns are in the gene. There are both "normal size" exons (140 bp, 200 bp) and large exons (> 1 kb in size). In human, the IRBP gene is on chromosome 10 at 10q11.2 to 10q21.1, as determined by in situ hybridization of our bovine gene probe to human chromosome squashes. Also, we have isolated a genomic clone from a human chromosome 10 specific library. The clone was sequenced partially and contains part of one exon and part of an intron. The IRBP gene has been mapped in dog to chromosome 4. In situ hybridization to tissue sections of the retina showed that the IRBP mRNA accumulates in the layer of photoreceptor perikarya and showed less signal over cone cell perikarya. This suggests that the IRBP gene is active in the rod photoreceptor primarily. We have searched the existing Genbank and PIR databases to examine for other sequences that might be similar to the bovine IRBP gene, mRNA and protein sequences. None have been identified yet. These cloned probes allow us to analyze the IRBP gene from normal individuals and those affected with genetic eye diseases.

I 012 REGULATION OF GUANINE NUCLEOTIDE-BINDING PROTEINS, Martha Vaughan and Joel Moss, Lab of Cellular Metabolism, National Heart, Lung, and Blood Institute, Bethesda, MD 20892.

Numerous hormones, neurotransmitters and drugs exert their effects by interacting with cell surface receptors coupled through guanine nucleotide-binding (G) proteins to intracellular enzymes. Examples of such transmembrane signalling systems include the adenylate cyclase system, in which G proteins, G_s and G_i, mediate effects of stimulatory and inhibitory receptors, respectively, and the visual excitation complex, in which the photon receptor rhodopsin is coupled through G_t (transducin) to a cyclic GMP phosphodiesterase. The G proteins are heterotrimers composed of α (39-47 kDa), ß (35-36 kDa), and γ (7-11 kDa) subunits. The α subunits bind guanine nucleotides and possess intrinsic GTPase activity; the ßγ subunits facilitate interaction of α with receptor. The agonist·receptor complex promotes G protein activation and signal propagation by accelerating exchange of GDP for GTP on the α subunit and dissociation of the active α·GTP species from ßγ. Hydrolysis of bound GTP produces G_α·GDP and terminates the signal. The α subunits of different G proteins exhibit functional, structural, and immunological similarities. Comparison of deduced amino acid sequences reveals extensive similarity in regions believed to be involved in guanine nucleotide binding and GTP hydrolysis as well as highly divergent sequences that may confer specificity for receptor and effector interaction. The activity of the α subunits is altered by ADP-ribosylation catalyzed by the bacterial toxins, choleragen (cholera toxin) and pertussis toxin. ADP-ribosylation of $G_{s\alpha}$ by choleragen, which results in the activation of adenylate cyclase, is enhanced by another guanine nucleotide-binding protein, known as ADP-ribosylation factor or ARF. Three forms of ARF purified from bovine brain, in addition to enhancing the ADP-ribosylation of $G_{s\alpha}$, stimulated choleragen-catalyzed ADP-ribosylation of unrelated proteins and simple guanidino compounds (e.g., arginine), auto-ADP-ribosylation of the toxin A_1 subunit, and NAD hydrolysis. All of these effects required GTP or an analogue; GDP, its analogues, and ATP analogues were ineffective. Kinetic data are consistent with the conclusion that ARF interacts directly with choleragen in a GTP-dependent fashion to increase its catalytic activity at low substrate concentrations. Since both ARF, the allosteric activator, and the toxin substrate $G_{s\alpha}$ are guanine nucleotide-binding proteins, it appears that the pathological activation of adenylate cyclase by choleragen involves a G protein cascade. The physiological role of ARF as well as possible relationships to the G protein α subunits and/or the ras oncogene products remain to be determined.

Molecular Biology of the Eye: Genes, Vision & Ocular Disease

Special Lecture

I 013 PROTEIN EVOLUTION

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The availability of large numbers of amino acid sequences in data banks is providing new insights into evolution in general and the structure-function aspect of proteins, in particular. It is becoming increasingly clear that a small number of archetypal polypeptides was expanded by the general route of "duplication and modification" to give the vast inventory of proteins in existence today. In addition to the many well known families of proteins, a large number of unexpected relationships has emerged as a result of the computer-assisted searching of sequence banks. This same approach has uncovered many instances, also, of exon shuffling. The picture that is emerging is one where a set of enzymes and other critical proteins was set in place early, long before the divergence of prokaryotes and eukaryotes. Since that time, some genuinely new protein-types have arisen, including many peculiar to eukaryotes. Enough data are now available that some realistic dates can be attached to when certain episodes in biochemical evolution must have occurred.

Evolution of Ocular Proteins and Genes

I 014 MOLECULAR BASIS OF THE VISUAL CYCLE, C. David Bridges and Shao-Ling Fong, Dept. of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, Indiana 47907.

Interstitial retinol-binding protein (IRBP) is a large transport protein that provides the extracellular link between rhodopsin in the photoreceptors and the isomerizing system in the pigment epithelium. Like rhodopsin, it is synthesized by the photoreceptors. Five overlapping clones from a human retina cDNA library have been isolated and sequenced to provided a 4240-base nucleotide sequence that encodes 1262 amino acid residues (M_r = 136,600) together with untranslated 5' and 3' segments. The 5' end has been verified from the genomic DNA sequence. The mRNA is approximately 5,200 base pairs long. Four duplicated regions occur sequentially in the conceptually translated amino acid sequence, leaving 5 unaligned at the N-terminus and 42 at the C-terminus. There are several short deletions and insertions. These segments may be organized into folding domains associated with retinol binding. A predicted alpha-helical region is found at the end of each segment. The sequences of each segment are 33-38% identical, with SD's of 20.9 - 29.8 from the mean scores for randomized alignments. The results suggest evolution by gene duplication from a 34,000 M_r ancestral protein about 600-800 million years ago, before emergence of the vertebrates.

I 015 MOLECULAR EVOLUTION OF α-CRYSTALLIN, Wilfried W. de Jong, Jack Leunissen, Wiljan Hendriks and Hans Bloemendal, Department of Biochemistry, Center of Eye Research, University of Nijmegen, P.O.Box 9101, 6500 HB Nijmegen, The Netherlands.

α-Crystallin is related to the small heat shock proteins (HSPs) (1). Comparison with all currently available sequences of small HSPs reveals that the ancestral α-crystallin gene originated from a single complete copy out of the multigene small HSP family. Homology has been best conserved in the C-terminal parts of the proteins, encoded by the two 3′ exons of the α-crystallin genes. Evidence for homology can, however, also be found in the N-terminal sequences of several HSPs. The major region of homology between α-crystallin and small HSPs is also shared with the egg protein p40 of the blood fluke Schistosoma mansonii (2). The function of the homologous domain is probably homeostatic, conferring protection by associating with other cellular components (1) or by their thermodynamically stable structures (3).

The primordial α-crystallin gene has duplicated into the ancestral αA and αB genes, before the radiation of the vertebrates, and both have apparently remained single-copy genes ever since. The αA and αB genes are differentially expressed during ontogenesis and in different vertebrates. The average rate of evolutionary change of the α-crystallin subunits is slow: 3 amino acid replacements per 100 residues in 100 million years. In the subterranean mole rat, however, which has no visually functioning eyes anymore, this rate has increased fourfold, reflecting a release of functional constraints (4). During the evolution of α-crystallin there has been a marked avoidance of changes in charge. Whereas 33% of amino acid replacements in the evolution of proteins should involve a change in charge, this has been less than 10% in the evolution of αA and αB.

Rodents and some other, disparate mammalian species have a minor elongated α-crystallin chain, αA^{ins}, with a 23-residue insert between positions 63 and 64 of an otherwise normal αA chain. The αA^{ins} chain has been shown, in rodents, to be the result of alternative splicing of the primary gene transcript (5). αA^{ins} seems to be structurally and functionally equivalent to normal αA chains. It almost seems a paradox that the sudden insertion of 23 residues into such a slowly evolving protein, modelled through several hundreds of million years of evolution, yields a viable product that has been maintained in several mammalian lineages during more than 70 million years.

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I 016 **THE COLLAGEN GENE FAMILY AND ITS EVOLUTION**, Gabriel Vogeli, Paul S. Kaytes, Linda Wood, Molecular Biology Research, The Upjohn Company, Kalamazoo, MI 49001

The vertebrate eye is a complex organ in which at least four types of collagens interact to create the physical shape of the optical apparatus. The regulatory mechanisms leading to this three dimensional structure that allows light to be focused onto the retina must therefore control collagen synthesis in a very precise way. The analysis of such temporal and spatial control mechanisms will provide major insights into vertebrate morphogenesis.

Collagens, the most abundant animal proteins, are the major constituents of the extracellular matrix. Collagens have been described for the most ancient forms of animals like the sponges and the coelenterates. The first collagens found might have been composed solely of Gly-X-Y repeats; such primitive collagens might be still found in the sponges and the nematodes. The evolutionary complexity of collagen increased dramatically at the beginning of the vertebrate expansion around 500 million years ago. At this time, fibrillar collagens (type I and type III) together with the cartilage collagens elaborated the typical vertebrate skeletal apparatus. During the evolution of these collagens, a 54 bp ancestral structural gene was assembled into larger genetic units without the concomitant removal of intervening sequences. The regulatory elements of a few collagen genes have been analyzed in detail and some general rules for collagen promoters and translation initiation sites emerged. In addition, special biosynthetic mechanism guarantee that an intact functional collagen triple helix is synthesized, assembled and excreted into the extracellular space. Many posttranslational modifications guarantee the correct assembly into the mature collagen and thus into the three dimensional structure of tissues and organs. Many mutations that affect the collagen triple helix will produce an organism that is defective.

The major breakthrough in the evolution of multicellular organisms from unicellular organisms was the development of an extracellular matrix. In addition, substantial evolutionary advantage was introduced when the coelenterates assembled the extracellular collagens, together with other molecules, into a distinctive extracellular structure called the basement membrane. This selective advantage was so powerful that very few animal species survived through geological time periods without an extensive system of collagenous basement membranes. Since the advent of the coelenterates, cells in contact with a basement membrane have been able to undergo the most extensive spectrum of differentiation compared to any other class of cells within any organism. Hence we speculate that the presence of a collagenous basement membrane has been the major driving force throughout most of the animal evolution.

I 017 THE ORIGINS OF CRYSTALLINS. Graeme J. Wistow and Joram Piatigorsky, LMDB, National Eye Institute, NIH, Bethesda, MD 20892.

The lens is a specialized tissue whose primary components are soluble proteins, the crystallins. As protein and nucleic sequences have accumulated the evolutionary relationships of these proteins have been revealed. α-Crystallin and the βγ-crystallins, which appear to be truly lens specific, are related to, but distinctly diverged from, non-lens proteins. The α-crystallins are strikingly similar to the small heat shock proteins while β- and γ-crystallins are structurally related to a bacterial spore coat calcium-binding protein. These proteins seem to have arisen by gene duplication, divergence and specialization. The evolutionary history of the other major crystallins, those specific to different taxonomic groups, is markedly different. They are closely similar to several enzymes, lactate dehydrogenase (ε), argininosuccinate lyase (δ), enolase (τ) and members of the aldehyde reductase superfamily (ρ). In the same way, the lens protein of the squid may be related to gluatathione S-transferase.

It now appears that at least some of these crystallins are actually the products of the same genes as the related enzymes. This suggests that normal enzymes have in some species acquired an additional role as major lens proteins, presumably by modification to their gene promoters.

The duck lens is an abundant source of ε/LDH, δ/ASL and τ/enolase. In our laboratory cDNAs for two duck δ-crystallins and duck lens τ/enolase have been obtained and sequenced. Work is in progress to analyse the expressed products of these cDNAs and to isolate the promoter regions of the respective genes.

The pattern of occurence of crystallins in different taxa suggests an interesting evolutionary history. This is particularly so for mammals whose eyes have probably experienced unusual selective pressure due to large scale changes in habit.

Gene Expression and Differentiation - I

I 018 MULTIPLE ELEMENTS CONTROL DEVELOPMENTAL REGULATION OF γ-CRYSTALLIN GENE EXPRESSION. Martin L. Breitman[1,2], Si Lok[1,3], Mark Tini[1,2], Daphne Goring[1,3], Susan O. Meakin[1,2] and Lap-Chee Tsui[1,3]. (1) Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada, M5S 1A8. (2) Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5. (3) Department of Medical Genetics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8.

Our laboratory is interested in the molecular mechanisms regulating γ-crystallin gene expression in the eye lens. In previous studies, we have shown that the 5' flanking sequences of representative mouse and human γ-crystallin genes contain lens-specific promoter activity as well as sufficient information to direct appropriate developmental expression of reporter genes in the lenses of transgenic mice. For the mouse γ2 gene, this information has been localized to sequences between -226 and +45. Detailed mutation analysis of this interval has revealed that it is functionally complex and comprised of at least three operationally defined regions, two of which are essential for promoter function. Region I (-67 to +8) is highly sensitive to mutation and encompasses a 44 bp segment that is highly conserved among different γ-genes. Region II (-189 to -162) is also critical for promoter activity and corresponds to a portion of an enhancer element. Region III (-162 to -67) is essentially not required for promoter function as defined by deletion analysis, although a linker scanning mutation between -137 and -128 completely abolishes promoter activity. At least two enhancer elements within the upstream sequences can be defined; one between -226 and -123, and a second between -392 to -278. The latter element (-392 to -278) is apparently not essential for appropriate developmental regulation in vivo, but may serve to facilitate transcriptional activation during development. In contrast, the proximal element, (-226 to -123) which is critical for activity, specifically binds one or more nuclear proteins recovered from lens cells of 14 day-old chick embryos.

I 019 ACTIVATION OF THE Alpha A-CRYSTALLIN PROMOTER IN THE LENS. Ana B. Chepelinsky, Bernd Sommer, Eric Wawrousek and Joram Piatigorsky. Laboratory of Molecular and Developmental Biology, National Institutes of Health, Bethesda, MD 20892.

We have been investigating the regulatory ability of the murine αA-crystallin promoter by fusing the sequence between positions -366 and +46 to the bacterial chloramphenicol acetyltransferase (CAT) gene in the pSVOCAT vector. Our initial experiments showed that this αA-crystallin gene fragment contains a lens-specific promoter when tested in embryonic chicken lens epithelia (1), rabbit lens epithelial cells (2) and transgenic mice (3). When fused to the SV40 large T-antigen gene, the resulting transgene abolished lens fiber formation in mice (4).

Further analysis of the murine αA-crystallin 5' flanking sequences in transient expression assays in explanted chicken lens epithelia demonstrated the existence of two interacting cis regulatory elements--one present in a distal domain between nucleotides -111 to-84, and the other in a proximal domain -88 to +46. Sequence -88 to -60 contains a functional element of the proximal domain. These cis regulatory elements interacted in vitro with factors present in chicken lens nuclear extracts. A DNA fragment corresponding to the sequence -55 to -111 was retarded when electrophoresed in an acrylamide gel after incubation with the nuclear extract. Methylation interference experiments indicated that bases present in both domains are involved in the interaction with protein factors. When each domain (-55 to -83 and -84 to -111) was tested independently in a gel retardation assay, both bound nuclear factors that appeared to be different. Mutational analysis and methylation interference experiments using oligonucleotides indicated that bases between -72 and -79 of the proximal domain and -90 to -103 of the distal domain are involved in the protein interaction. An oligonucleotide of the distal domain with substitutions at positions -108 and -109 lost is ability to activate the proximal domain of the αA-crystallin promoter, while mutations at positions -96 and -97 had no effect when tested in explanted chicken lens epithelia. Finally, mice carrying a hybrid transgene containing αA-crystallin sequences -111 to +46 fused to the CAT gene established that this short promoter sequence is sufficient to activate gene expression in the lens.

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I 020 REGULATION OF THE CHICKEN δ-CRYSTALLIN EXPRESSION: INTRAGENIC ENHANCER AS THE MAJOR DETERMINANT FOR LENS-SPECIFICITY, Hisato Kondoh, Koji Goto and Shigeo Hayashi, Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan.

Introduction of the molecular-cloned δ1-crystallin gene into mouse cells in primary culture (1) and into transgenic mice (2) resulted in proper regulation of the gene with respect to tissue specificity, indicating that DNA elements essential for lens-specific gene regulation are located within the introduced DNA sequence. Since deletion analysis indicated that the region upstream of position -100 is not necessary for lens-specific expression (3), and since the promoter of the gene did not show tissue specificity, we examined various segments of the δ1-crystallin gene for a tissue-specific enhancer activity by placing each segment downstream of a heterologous transcriptional unit coding for chloramphenicol acetyltransferase (CAT), and by transfecting chicken tissues in primary culture. We found that a segment spanning the third intron bears a strong lens-specific enhancer activity (4). This "δ1-crystallin enhancer" activates transcription from the δ1-crystallin promoter 20 to 40 fold in lens cells and to various degrees with other promoters. Deletion analysis of the enhancer region indicated that it covered nearly 1 kb but did not indicate clearcut boundaries. For its enhancer effect the core region of 120 bp and associations with certain adjoining regions were required. Removal of the enhancer from the gene totally abolished δ1-crystallin expression, and re-insertion of the enhancer in either upstream, internal or downstream positions restored expression. We conclude that the δ1-crystallin enhancer is the major determinant for lens-specificity of δ1-crystallin expression.

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I 021 A COMPARATIVE SURVEY OF CRYSTALLIN GENE EXPRESSION. J. Piatigorsky, D. Parker, E. F. Wawrousek, C. A. Peterson, J. F. Klement, J. Roth, A.B. Chepelinsky, T. Borras and G. Thomas. Laboratory of Molecular and Developmental Biology, National Eye Institute, Bethesda, MD 20892.

A brief overview will be given showing that the crystallin gene families are expressed preferentially at a very high rate or exclusively in the lens in a species-independent fashion. Nonetheless, no lens-specific consensus sequence controlling gene activity has been identified. Conversely, even the extremely similar, duplicated, tandemly linked chicken δ1 and δ2-crystallin genes are expressed differentially both quantitatively and, possibly, qualitatively in the embryonic lens. To identify cis-acting control elements, we have monitored the ability of 5' flanking regions of different crystallin genes fused to the bacterial chloramphenicol acetyltransferase (CAT) gene to direct CAT expression when transfected into lens epithelial cells from chicken embryos. Although other internal or 3' sequences may have major roles in regulating crystallin gene expression, considerable tissue-specific activity was directed from the promoter regions of the chicken and mouse αA- and the chicken βB1-crystallin genes; some lens-preference was also obtained with the chicken δ1-crystallin promoter. Our experiments indicate that *crystallin promoters contain various regulatory elements. For example, even the* corresponding αA-crystallin genes from mice and chickens appear to include different regulatory sequences despite their overall similarity, and deletion tests using the δ1-crystallin promoter provide evidence for both positive and negative control elements. Competition experiments using gel retardation as an assay suggest that the chicken δ1-and βA3/A1-crystallin promoters bind different nuclear proteins. Further experiments combining structural and functional assays should eventually resolve a spectrum of crystallin transcription factors.

I 022 *CONTROL MECHANISM OF THE ALPHPA-CRYSTALLIN GENE EXPRESSION*, Kunio Yasuda, Masayuki Takeuchi and Masahi Kitamura, Dept. of Biophysics, Fac. of Science, Kyoto University, Kyoto 606, Japan.

Lens cells of eye tissues of the vertebrates are terminally differentiated cells, which specifically synthesize a large amount of crystallins. Crystallins are highly conserved proteins and are classified into four groups, α,β,γ and δ. Crystallin genes are expressed at a specific time and site during lens development. To study the tissue-specific expression of the αA-crystallin gene at molecular level, we cloned the chicken αA-crystallin gene and introduced the gene in a variety of primary cultured cells to examine its expression. When αA-crystallin promoter sequence fused to the bacterial CAT gene was tested, the fusion gene with 5' flanking sequence is expressed only in lens cells. Deletion experiments showed that the sequence between -245 and -90 was essential for lens-specific expression. If the fusion gene was co-transfected with these -245 to -90 sequences, its expression was reduced to 50 % when the molar ratio of the competitor sequence to the gene was 150 to 1. This result showed the presence of a positive regulatory factor in lens cells binding specifically to the -245 to -90 sequence. To indicate the presence of such a factor in lens cells, DNA-nuclear protein interaction was studied with the -245 to -90 sequence. With the gel retardation assay, a DNA binding protein which specifically interacts with the -160 to -90 sequence was detected in nuclear extracts from lens cells. To study further the molecular character of the DNA binding protein, a nuclear protein blot from various tissues was probed with different labeled DNA sequences. This showed that the 61 kD protein in lens cells specifically interacts with the sequence between -160 and -90.

Gene Expression and Differentiation - II

I 023 IDENTIFICATION AND EXPRESSION OF DROSOPHILA PHOTOTRANSDUCTION GENES, Craig Montell[1], Drzilav Mismer[1], Mark E. Fortini[1], Charles S. Zuker[2] and Gerald M. Rubin[1], [1]Department of Biochemistry, University of California, Berkeley, CA 94720, [2]Department of Biology, University of California, San Diego, CA 92093.

The fruitfly, *Drosophila melanogaster*, is an excellent system in which to both study the roles of previously identified photoreceptor cell proteins and to isolate and characterize the roles of genes and proteins important in phototransduction that have not yet been identified in any metazoan system. There are two important advantages of studying phototransduction in the fruitly: 1) many of the relevant genes have already been genetically identified 2) DNA sequences can be stably and efficiently introduced into the genome by P-element mediated germline transformation. Therefore, it is possible to identify the DNA sequence encoding a genetically defined locus by complementing the mutant phenotype following introduction of the wild-type gene into the genome by germline transformation. Using this approach, we have identified two genes, *trp* (1) and *ninaC*, by complementing the mutant phenotypes. Both genes are expressed beginning late in development and encode proteins localized specifically to the photoreceptor cells. The *ninaC* proteins share significant homologies to two types of proteins which define two nonoverlapping domains. One domain shares amino acid homology to protein kinases and the other to all of the globular head region of the myosin heavy chain. The *trp* protein is not homologous to previously sequenced proteins but displays a number of very striking internal repeats. Using a different approach, we have identified two opsin genes, Rh3 and Rh4, expressed specifically in the R7 ultraviolet sensitive subset of photoreceptor cells (2, 3). Thus, a total of four related Drosophila opsin genes have now been isolated. Each of these opsin genes is expressed in a nonoverlapping subset of photoreceptor cells in the fly's compound eyes. Experiments directed at identifying the DNA sequences required for the proper spatial and quantitative expression of each of the opsin genes will be described.

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I 024 PROTO-ONCOGENE EXPRESSION DURING IN VITRO DIFFERENTIATION OF EMBRYONIC CHICKEN LENS EPITHELIAL EXPLANTS. Peggy S. Zelenka and Luke A. Pallansch, Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

Explants of embryonic chicken lens epithelia differentiate in vitro to form lens fiber cells when cultured in the presence of IGF-I or related substances. We have investigated the expression of several proto-oncogenes during this process using specific DNA probes for proto-oncogene mRNA's and a sensitive nuclear "run-on" transcription assay. Of seven proto-oncogenes investigated, three are very actively transcribed in the rapidly dividing cells of the central lens epithelium: c-myc, c-fos, and p53. All three code for short-lived nuclear proteins thought to be involved in regulation of the cell cycle. Surprisingly, cell cycle arrest and differentiation of the lens cells are not accompanied by changes in the rates of transcription of these genes. Nevertheless, specific changes in the cytoplasmic levels of c-myc mRNA are observed during differentiation. Levels of c-myc mRNA transiently rise about 10-fold during the first few hours of differentiation, as cells withdraw from the cell cycle. The absence of concomitant changes in c-myc gene transcription indicates that this transient increase in c-myc mRNA levels is post-transcriptionally regulated. A similar increase in c-myc mRNA levels is produced by inhibitors of the lipoxygenase pathway of arachidonic acid metabolism, suggesting that some product of this pathway may exert a negative regulatory effect on c-myc expression. Furthermore, initiation of differentiation in cultured explants by IGF-I related substances is correlated with the loss of certain lipoxygenase pathway metabolites and the cessation of phosphatidylinositol turnover. These findings provide a possible link between arachidonic acid metabolism and proto-oncogene expression, and underscore the importance of post-transcriptional controls during lens cell differentiation.

Molecular Basis of Ocular Disease - I

I 025 STRUCTURAL AND FUNCTIONAL STUDIES OF S-ANTIGEN (48K PROTEIN) IN UVEITIS, Larry A. Donoso, M.D., Ph.D., Wills Eye Hospital, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107.

Inflammatory diseases of the eye are a significant cause of visual handicap in the United States and throughout the world. S-antigen (48K protein) is a photoreceptor cell protein intimately involved in the visual process and highly pathogenic for the induction of experimental autoimmune uveitis (EAU). EAU is a severe inflammatory disease which eventually results in the complete destruction of the photoreceptor cell layer of the retina. T-cells appear to play a major role in the pathogenesis of EAU since the disease develops following the adoptive transfer of lymph node cells or spleen cells from animals previously immunized with S-antigen. T-cells have also been implicated in some forms of uveitis in humans, such as sympathetic ophthalmia, and in some patients T-cell responses to S-antigen can be elicited.

In order to identify specific sites in bovine S-antigen responsible for its pathogenicity, we first determined the amino acid sequence (T. Shinohara, this symposia). These studies indicate that the pathogenic region of bovine S-antigen is located within one large 122 amino acid containing cyanogen bromide peptide fragment, designated peptide C5. This fragment also contains the monoclonal antibody MAbA9-C6 binding site, a monoclonal antibody known to inhibit or delay the onset of EAU, as well as a potential cytotoxic T-cell recognition site. Using synthetic peptides, one such pathogenic site has been characterized. These studies localized this site to a small, well-characterized region of bovine S-antigen corresponding to amino acid positions 303 to 320. The relevance of these findings to human disease is indicated by the finding that the amino acid sequence of human S-antigen is this and the surrounding region is identical to bovine S-antigen (T. Shinohara). Additional pathogenic sites are at present under investigation as well as the role of other peptides in various B- and T-cell mediated responses.

A knowledge of the amino acid sequence of S-antigen may provide important clues as to the etiology of certain forms of inflammatory diseases by the concept known as molecular mimicry. For example, a six amino acid sequence in hepatitis B virus polymerase (HBVP) is homologous with a six amino acid sequence in the encephalitogenic site of myelin basic protein (MBP), responsible for the induction of experimental autoimmune encephalomyelitis (EAE). Immunization of rabbits with a synthetic peptide corresponding to HBVP elicits antibody responses, and central nervous system lesions reminiscent of EAE illustrating that a viral peptide can induce cross-reactive antibody responses and generate inflammatory responses at the location of the self protein, molecular mimicry. This concept will be discussed in greater detail.

I 026 ONCOGENIC MUTATIONS OF THE RETINOBLASTOMA GENE, Thaddeus P. Dryja, M.D., Taylor R. Smith Laboratory, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston 02114.

Retinoblastoma is a cancer that arises from the retinas of children, usually under the age of 5 years. Approximately 30 to 40% of patients with retinoblastoma carry a heritable tumor-predisposing mutation at the "retinoblastoma locus" which is within human chromosome 13, band q14. These mutations can be passed on to future generations, and the individuals who carry a mutant allele at the retinoblastoma locus have a 90% chance of developing the cancer. It is of clinical value to ascertain which members of families with hereditary retinoblastoma carry a tumor-predisposing mutation. With this goal in mind, members of my laboratory undertook this project.

Dr. S. Friend, I, and our colleagues have isolated by molecular cloning a cDNA fragment corresponding to the mRNA transcript of the retinoblastoma gene.[Nature 323:643-646, 1986] Beginning with this cDNA fragment, Dr. J. Wiggs, Ms. J. Rapaport, Dr. D. Yandell, and I have isolated recombinant bacteriophage containing human genomic sequences derived from the retinoblastoma gene. Over 30 distinct bacteriophage have been characterized. The human DNA sequences within these bacteriophage span a continuous genomic DNA segment over 200 kilobases in size within the retinoblastoma locus. They contain over 90% of the sequences found in the mRNA transcript. Within this genomic region, we have identified 5 sites of DNA polymorphism (restriction fragment length polymorphism). These polymorphic DNA sites are useful for tracing the inheritance of alleles at the retinoblastoma locus through a family with hereditary retinoblastoma.

Using a set of 20 families with hereditary retinoblastoma, we observe that approximately 90% of hereditary retinoblastoma families are informative with at least one of these polymorphisms. In three families with hereditary retinoblastoma, we identified deletions involving the retinoblastoma locus. In each of these families, a specific deletion is common to all affected family members or obligate carriers. Since it is likely that such deletions represent the tumor-predisposing mutations in these families, the differentiation of family members at high risk and those with no increased risk for retinoblastoma is very accurate. Among the remaining 17 families, we observed one kindred where the retinoblastoma trait does not segregate with the intragenic sites of DNA polymorphisms. The absence of co-segregation could be due to intragenic recombination between the mutation predisposing to the tumor and the sites of the DNA polymorphisms. Alternatively, it could indicate that in some families, hereditary tumor-predisposition is governed by a second tumor-predisposition locus that is not tightly linked to the cloned retinoblastoma locus.

Our data demonstrate the clinical usefulness of cloned sequences from the retinoblastoma gene. They represent the use of a human cancer gene for the diagnosis of individuals with a high cancer susceptibility. Because the DNA polymorphisms are highly informative, the retinoblastoma locus may now be valuable for the study of other genetic loci within or near 13q14, such as the Wilson's disease locus.

I 027 IDENTIFICATION OF MUTATIONS IN THE PUTATIVE RETINOBLASTOMA GENE. Brenda L. Gallie, James M. Dunn, Audrey Goddard, and Robert A. Phillips, Hospital for Sick Children Research Institute, University of Toronto, Toronto, Canada M5G 1X8.

Retinoblastoma (RB) tumors develop when both alleles of a gene (Rb1) are mutated and unable to function normally. In some cases, one mutant allele in the germline results in a hereditary predisposition to development of RB tumors in infancy, and osteosarcoma (OS) tumors in adolescence. In other cases, with no hereditary tendency, both alleles of the same gene are mutated in the somatic retinal cell that becomes malignant. Recently, Friend et al.[1] reported the cloning of a gene, 4.7R, with some properties expected of the Rb1 gene. Since we found that 80% of RB and OS tumors from RB patients showed normal genomic DNA restriction patterns and normal size and amounts of mRNA on Northern blots using the 4.7R gene probes, a possibility remained that the 4.7R and Rb1 were separate but closely linked genes that were both involved in deletion mutations.

Detailed characterization of three RB tumors now supports the identification of 4.7R as the Rb1 gene. One RB tumor has an insertion of approximately 19 kb in an intron near the 5' end of 4.7R. Two unrelated RB tumors showed abnormalities in mRNA transcripts detected by RNAse protection, although Northern blot analysis was normal. The patient with bilateral RB showed the RNAse protection abnormality in both constitutional and RB tumor cells, but both of his parents had normal RNA. This appears to be a new germline mutation. The patient whose unilateral RB tumor showed an RNAse protection abnormality had normal constitutional RNA, confirming that the mutation in the tumor arose in a somatic cell.

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I 028 MOLECULAR AND GENETIC ANALYSIS OF THE MOUSE EYE LENS OBSOLESCENCE (*Elo*) AND THE CATARACT^{FRASER} MUTANTS. Lap-Chee Tsui[1,2], Paul Quinlan[1,2], Lidija Covic[1,2], Sen–ichi Oda[3], Roger Paterson[2,4] and Martin Breitman[2,4]. (1) Department of Genetics , Hospital for Sick Children, Toronto, Ontario, Canada; (2) Departments of Medical Genetics and Medical Biophysics, University of Toronto; (3) Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan; (4) Division of Cancer Research, Mount Sinai Hospital, Toronto, Ontario, Canada.

Eye lens obsolescence (*Elo*) is a fully penetrant, autosomal dominant eye disorder in mice. The lens defect is first detectable at 12 days of gestation, when the primary fiber cells of the mutant lens fail to elongate properly. The secondary fiber cells also develop abnormally, leading to a general deformation of the lens and microphthalmia noticeable at birth. No other ocular or non-ocular tissue appears to be affected in the mutant mice. Previous studies showed that the *Elo* mutation is located on chromosome 1, at a site near the *Len-1* locus defined by a set of polymorphic γ-crystallin proteins. These lens specific proteins are encoded by a cluster of six closely related genes, which are differentially regulated during lens development. To investigate the etiology of *Elo*, we have analyzed the steady state levels of γ-crystallin transcripts in the mutant mice and the linkage relationship between the Elo locus and the γ-crystallin genes. Our data showed that, while the γ-crystallin mRNA levels are preferentially reduced in the mutant eyes, the mutation does not seem to map within the tandemly linked γ-crystallin gene cluster. The latter conclusion was derived from genetic linkage analysis of restriction fragment length variants associated with the g-crystallin genes in normal and mutant mice. The latter study also suggest that the gene order is 5'-γ2-γ4-γ3-γ5-γ1-γ6-*Elo*. The distance between *Elo* and the γ6 gene is estimated to be approximately 1.4 centiMorgans (cM), whereas that between γ6 and the distantly linked γ2 gene is 2.7 cM. However, the γ-crystallin gene cluster is probably less than 150 kb in size, as indicated by our long range restriction mapping analysis using pulsed field gel electrophoresis. The detected recombination frequency is thus about 10 fold higher than the average in the mouse genome, suggesting the presence of recombination hot spots within the γ-crystallin gene cluster. The tight linkage between the γ-crystallin gene cluster and *Elo* also suggests that it is possible to clone the mutant locus by the chromosome jumping technique.

Cataract^{Fraser} is another dominant mouse mutation which results in reduced accumulation of all γ-crystallin transcripts in the lens. To investigate the temporal and spatial effects of this mutation on γ-crystallin gene expression, a bacterial β-galactosidase gene fused to the mouse γ2-crystallin promoter has been introduced into the mutant mice by matings between homozygous mutant and transgenic mice carrying the reporter gene. Comparison of the spatial distribution and levels of the bacterial enzyme in normal and mutant lenses revealed that, while the overall distribution of β-gal activity is similar between the two, there is a significant decrease of β-gal activity in the nuclear fiber cells of the mutant lens. Since this reduction is readily detectable in newborn animals and persists throughout the mutant lens development, it is probable that cataractogenesis in Cat^{Fraser} is the result of a general increase in the rate of mRNA turnover in the mutant lens fiber cells.

I 029 THE ACTION OF ONCOGENES AND PROTOONCOGENES IN THE LENS OF TRANSGENIC MICE, Heiner Westphal, M.D., Section of Mammalian Gene Regulation, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

My colleagues, Kathleen Mahon, Takafumi Nakamura, Ana Chepelinsky, Jaspal Khillan, Paul Overbeek, Joram Piatigorsky, and myself have studied oncogenesis in the lens. We constructed a hybrid transgene consisting of murine lens-specific αA-crystallin enhancer/promoter sequences fused to the coding sequence of the SV40 T antigens. This transgene, which we will call αT was inserted in the germline of mice and several αT strains were obtained. While neoplastic tumors of the lens have never been observed in the vertebrate, our transgenic mice carrying the αT gene developed lens tumors which eventually obliterated the eye cavity and invaded neighboring tissues. Transformed cells expressing SV40 large T antigen were seen in the lens as early as day 12 of development, the day of primary lens fiber differentiation. In adult mice, we observed tumors not only in the eye but also at ectopic sites, notably in the gingiva, the epithelia of the thymus, and in striated muscle. Both the lens tumors and the ectopic tumors express SV40 large and small T antigens. The accumulation of α, β, or γ crystallins in lens tumors is inversely proportional to the degree of neoplastic transformation exerted by the oncogene. They never occur in ectopic tumors. We therefore assume that ectopic tumors are due to a leakiness of the αA crystallin promoter and not to metastatic events. In a second project, my colleagues Jaspal Khillan, Fritz Propst, Marianne Oskarsson, Toichiro Kuwabara, George Vande Woude and myself have examined the genetic effects in the lens of a hybrid gene which contains the murine c-*mos* protooncogene under the control of the long terminal repeat of Moloney murine sarcoma virus. This hybrid gene scores highly positive in the 3T3 cell transformation assay but, as a transgene, has failed to cause any noticeable malignancies. However, three of the transgenic strains are characterized by a unique type of lens pathology. Shortly after birth, insufficient posterior elongation of differentiating lens fibers and lack of basement membrane secretion leads to breakdown of the posterior lens capsule. This, in turn, results in a posterior protrusion and swelling of lens tissue. In the course of the first three weeks after birth, globular lens cells begin to fill the entire anterior and posterior chambers of the eye. Concomitantly, there is massive overexpression of c-*mos* RNA in the lens. Hyperplasia or neoplasia of tissues have not been detected in any of the three transgenic strains. Rather, c-*mos* overexpression appears to affect distinct steps in lens fiber differentiation. Our current working hypothesis envisages that *mos* expression results in inhibition of proliferation of those lens epithelia which give rise to secondary fibers, thereby causing the described chain of events.

Molecular Basis of Ocular Disease - II

I 030 MOLECULAR GENETICS OF GYRATE ATROPHY, George Inana(1), Yoshihiro Hotta(1), Carmelann Zintz(1), Muriel Kaiser(1), Takashi Shiono(2), Akira Nakajima(3), David Barrett(4), J. Bronwyn Bateman(4), Robert Sparkes(4), Nancy Kennaway(5), and Richard Weleber(5), (1)National Eye Institute, NIH, Bethesda, MD 20892, (2)Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Japan, (3)Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan, (4)Department of Ophthalmology and Medical Genetics, UCLA School of Medicine,Los Angeles, CA 90024, (5)Department of Ophthalmology and Medical Genetics, Oregon Health Sciences University, Portland, OR 97201.

Gyrate atrophy(GA) is a blinding, autosomal recessive degenerative disease of the retina and choroid of the eye characterized by a generalized deficiency of the mitochondrial enzyme, ornithine aminotransferase(OAT). The knowledge of the underlying biochemical defect in GA enabled us to take a molecular genetic approach in studying this disease. First, we constructed and characterized a molecular probe for the human OAT in the form of a lambda gt11 cDNA clone. Analysis of the cDNA-derived OAT sequence revealed the presence of an OAT precursor containing a leader sequence similar to those found in other mitochondrial proteins of cytoplasmic origin. A differential hybridization analysis of the human genome using specific OAT cDNA-derived probes demonstrated the presence of one putative functional OAT gene and at least three other OAT-related genes indicating a gene family. The functional OAT and OAT-related gene sequences were mapped to chromosomes 10q26 and Xp11.2, respectively. A sequence analysis of OAT gene clones confirmed the chromosome 10 gene to be the functional gene and at least one of the X chromosome genes to be a pseudogene. Analysis of the OAT gene, mRNA, and protein in 20 GA patients using the OAT DNA and antibody probes demonstrated a GA case with a partial heterozygous deletion of the OAT gene, no OAT mRNA, and essentially undetectable level of OAT protein. The rest of the cases show grossly normal OAT gene and variably reduced levels of OAT mRNA and protein. In one of these cases the OAT mRNA level was shown to be half of normal, indicating expression of only one of the OAT gene alleles, and a point mutation was demonstrated in the expressed mRNA resulting in an amino acid change in the OAT protein. The results from these cases constitute the first real demonstration of the molecular genetic defect of OAT present in GA.

I 031 MOLECULAR INTERACTIONS OF CRYSTALLINS IN RELATION TO CATARACT.

C. Slingsby, H. Driessen, H. White, S. Mylvaganam, S. Najmudin, B. Bax, M.A. Bibby, P.F. Lindley, D.S. Moss and T.L. Blundell, Birkbeck College, Laboratory of Molecular Biology, Malet Street, London WC1 7HX, U.K. Yu. V. Sergeev and Yu. N. Chirgadze, Institute of Protein Research, Academy of Sciences of the U.S.S.R., Pushchino, Moscow Region, U.S.S.R. Eye lens cells are packed with proteins in order to achieve the required high refractive index. Irregular changes in refractive index sufficient to cause light scattering will contribute towards cataract. Packing is a function of the molecular interactions of crystallin monomers and oligomers and is determined by their surface properties. γ-crystallins are weakly interacting in dilute solution although some members of this closely related family undergo a phase transition whereby protein association is dramatically increased and are largely responsible for the loss of lens transparency in cold cataract. High resolution structures of three bovine γ-crystallins have been determined by x-ray diffraction techniques. All three molecules have very similar bilobal structures. However, each molecule has crystallized in a different space group utilizing its own selection of surface side chains as sites for molecular association. Of particular interest is the appearance of sulphur containing amino acids close to symmetry axes in all three crystalline proteins and the linking of ion pair networks between molecules in the lattice. Attention will be focussed on the amino acid replacements which characterize those γ-crystallins found in the densely packed lens nuclear region with the highest potential to cryoprecipitate. Some structural information on the β-crystallins, which form a superfamily with γ-crystallins, can be predicted from their sequence homology. Sequences of β-crystallin structural genes code not only for the folding of the individual polypeptides but also for their specific recognition of other subunits. The predominant basic subunit (βB2) on folding can form a stable homodimer which can be crystallized yet addition of other purified β-subunits of limited solubility can favour heteroligomer formation. Genetically controlled levels of interacting β-crystallin subunits may lead to an array of quaternary structures with the appropriate potential for molecular association and omission of crucial components could lead to uncontrolled aggregation forming light scattering centres. Knowledge of the hierarchy of crystallin interactions starting from folded domains to complex aggregates will contribute towards understanding the molecular control of protein packing in eye lenses. This will form a basis for studying the effects of altered proteins, altered levels of gene expression and altered metabolic environment on the transparent phase of the lens.

I 032 CHARACTERIZATION OF SEQUENCES DELETED FROM A PATIENT WITH CHOROIDEREMIA, DEAFNESS AND A SUBMICROSCOPIC DELETION, Robert L. Nussbaum, Richard A Lewis, David H Ledbetter, HHMI and Univ. of Pa., Philadelphia, and Baylor College of Medicine, Houston, Tx.

Choroideremia (tapetochoroidal dystrophy, TCD) (McK #30310) is an X-linked retinal dystrophy of unknown pathogenesis which causes progressive nightblindness and eventual central blindness in affected males. TCD has been previously mapped to Xq13-q21 by tight linkage to RFLP loci from this region. Two families with TCD, mental retardation and deafness were analyzed by molecular and cytogenetic techniques. In family XL-63, an interstitial deletion in Xq21 was visible and two linked DNA loci, DXYS1 and DXS72, were deleted. In family XL-45, a deletion was suspected based on phenotype but could not be confirmed by high resolution cytogenetic analysis, and no markers from the region Xq13-q21 were found deleted. We generated a λgt10 library enriched for sequences that might be deleted in family XL-45 using phenol-enhanced reassociation. Two single copy sequences, pJL68 and pJL8, were found deleted in both families. pJL8 was also deleted in a third patient with an Xq21 deletion, TCD and retardation but without deafness. To date, no alterations have been seen in TCD patients without retardation using these probes. A large scale restriction map is being generated in the region around pJL68 and pJL8 using pulsed-field gel electrophoresis and chromosome hopping techniques.

Phototransduction

I 100 INVESTIGATION OF RHODOPSIN-TRANSDUCIN INTERACTIONS BY SITE-SPECIFIC MUTAGENESIS. Roland R. Franke, Thomas P. Sakmar, Daniel D. Oprian and H. Gobind Khorana. Depts. of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139
The synthetic bovine opsin gene (1) was expressed in COS cells. After 11-cis retinal was added to the cells to reconstitute rhodopsin, the cells were solubilized and rhodopsin was purified by immunoaffinity adsorption. The visible absorption spectrum was indistinguishable from that of bovine rhodopsin. In order to investigate the interactions between rhodopsin and transducin, mutants were constructed with amino acid substitutions in the cytoplasmic loop linking the putative helices E and F (2). Expression and purification of the mutants was carried out as described above. Each of the mutants generated a wild-type visible absorption spectrum. The wild-type COS cell rhodopsin enhanced the light-dependent GTP hydrolysis activity of transducin with a specific activity identical to that of bovine rhodopsin (3). Two of the rhodopsin mutants activated transducin with the same specific activity as wild-type. One mutant (Lys248→Leu) did not activate transducin. Additional mutants are being constructed to elucidate the rhodopsin domain involved in transducin activation. Supported by grants from the ONR and NIH. TPS is supported by a fellowship from NIH and RRF is supported by a scholarship from the German Academic Exchange Service.

(1) Ferretti, L. et al (1986) Proc. Natl. Acad. Sci. USA 83, 599-603. (2) Hargrave, P. et al (1983) Biophys. Struc. Mech. 9, 235-244. (3) Oprian, D. D. et al Proc. Natl. Acad. Sci. USA (1987), submitted for publication.

I 101 PURIFICATION AND CHARACTERIZATION OF RHODOPSIN KINASE, P.A. Hargrave, K. Palczewski, and J.H. McDowell, Departments of Ophthalmology and Biochemistry, University of Florida, Gainesville, Florida, 32610-0284.
Rhodopsin (Rho) is the best characterized member of a family of homologus receptors which operate via G-proteins (the adrenergic receptors, muscarinic acetylcholine receptor, etc.). Rhodopsin kinase (RK) phosphorylates light-exposed Rho in a reaction important in deactivating the receptor. We have purified RK to homogeneity and find that it shares many properties with those of the β-adrenergic receptor kinase (ARK). RK may represent a model for understanding kinases which operate on this class of receptor proteins.
RK was purified to near homogeneity by affinity binding to light exposed rod cell outer segment membranes, ion exchange and hydroxyapatite chromatographies. This resulted in an 1100-fold purification of highly active RK with an overall recovery of 20%. RK, like ARK, has one subunit with Mr = 67 k as determined by gel filtration and SDS-PAGE. RK undergoes intramolecular autophosphorylation where both Ser and Thr are phosphorylated.
The kinetic parameters of the enzyme for (freshly bleached) Rho are Km=4 uM and Vmax=700 nmole/min/mg whereas for ATP Km=2 uM (about 20 times lower than comparable measurements for ARK [Benovic, et al., (1987) J. Biol. Chem. 262, 9026-9032]. GTP, on the other hand, is a very poor substrate (Km=1 mM, Vmax=10 nmole/min/mg). The kinase is very specifically inhibited by adenosine and its mono- and diphosphate derivatives, but not by most other adenosine derivatives. Compounds which change concentration during bleaching have only minor inhibitory effect on the kinase activity, with one exception--inositolmonophosphate can activate the kinase about 20% in 50-100 uM range of concentration.

I 102 IN VITRO EXPRESSION OF FUNCTIONAL BOVINE OPSIN USING A VIRAL EXPRESSION VECTOR, J.J.M. Janssen, W.J.M. van de Ven, W.A.H.M. van Groningen-Luyben, J. Roosien*, J.M. Vlak* and W.J. de Grip. University of Nijmegen, 6500 HB Nijmegen, *Agricultural University, 6709 PD Wageningen.
In vitro biosynthesis of full-length bovine opsin was accomplished by using a viral expression vector. Bovine opsin cDNA was inserted into the viral genome. Recombinants were isolated through immunohistochemical screening of infected cultured cells. Upon infection with a recombinant virus, $1x10^6$ cultured cells produced up to 3 ug opsin. The heterologous synthesized opsin was recognized by both poly- and monoclonal antisera which react with N- and C-terminal epitopes. Upon regeneration with 11-cis retinal a functional, hydroxylamine-stable, photosensitive pigment was formed.

I 103 IDENTIFICATION OF A BOVINE RETINAL PHOSPHATASE

Brenda Oppert and Dolores J. Takemoto, Department of Biochemistry, Kansas State University, Manhattan, KS 66506.

Soluble proteins isolated from bovine retina contain a phosphatase capable of dephosphorylating bovine rhodopsin. A 40,000 x g supernatant fraction from a total bovine retinal homogenate was further purified by anion exchange chromatography on DE-53 followed by high performance liquid chromatography on a TSK-3000 column. SDS-polyacrylamide gel electrophoresis and silver staining of the major protein HPLC fraction with phosphatase activity yielded bands of approximately 68, 67, and 65 Kd. This pattern is similar to that of the neural phosphatase, phosphatase 2b (calcineurin). Comparison of the two-dimensional peptide maps of this retinal phosphatase to that of purified bovine brain phosphatase 2b indicate that these are almost identical. The participation of phosphatase 2b in the vision cascade and its possible role in retinal degenerative diseases will be investigated. Supported by NEI-EY05623.

I 104 IDENTIFICATION BY CROSS-LINKING OF RHODOPSIN AS THE ROD OUTER SEGMENT (ROS) COMPONENT TO WHICH FGF BINDS.

Jean Plouet and Marie Dominique Loret, Cancer Research Institute, University of California at San Francisco, CA 94143 and U 86 INSERM, 15 rue de l'Ecole de Medecine, Paris, 75006.

Previous studies have shown that FGF binds in a light-dependent fashion to ROS membranes. In the present study, we have identified the ROS membrane components that interact with FGF using covalent cross-linking techniques. Under appropriate conditions, and provided that ROS membranes were illuminated, ^{125}I FGF was cross-linked, using disuccinimidyl suberate to a major component species with apparent molecular masses of 53 kDa and a minor one with a molecular weight of 90 kDa. These molecular weights correspond to that of FGF rhodopsin monomer and dimer complexes. Scatchard analysis of the binding data gave an apparent KD of 10 nM. Further evidence that FGF binds to monomeric and dimeric forms of rhodopsin were provided by the absence of cross-linked ^{125}I FGF rhodopsin complexes when the reaction was done under dark conditions, and by the fact that the SAg 48 kDa protein strongly inhibits the binding of FGF to illuminated ROS membranes, but not when the reaction was done under dark conditions. In parallel experiments, the α subunit of transducin had no effect on FGF binding to illuminated ROS membranes. FGF also increased GTP binding to illuminated ROS membranes. These results therefore suggest that FGF might act as a modulator of the visual transduction process through its ability to interact with rhodopsin and by preventing the interaction of SAg 48 kDa with rhodopsin.

I 105 CHARACTERIZATION OF A MOUSE TRANSDUCIN α SUBUNIT GENOMIC CLONE,

Carol J. Raport, Beverley Dere and James B. Hurley,
University of Washington, Seattle, WA 98195.

A mouse genomic library was screened with a mixture of bovine rod and cone transducin cDNA clones as probes. Five positive clones were identified; three strongly hybridized with the rod transducin probe and two strongly hybridized with the cone transducin probe. Sequencing of approximately 80% of the coding region of one of the clones has revealed a 99.6% identity between its derived amino acid sequence and that of bovine rod transducin, while the nucleic acid sequences are ~90% identical. Thus far six introns have been identified in the gene, three of which are in the same location as introns found in a Drosophila G protein genomic clone also characterized in our laboratory. Presently the upstream region of the mouse transducin clone is being analyzed to test whether it contains regulatory sequences sufficient for directing retina-specific gene expression.

I 106 STRUCTURAL ANALYSIS OF MOUSE S-ANTIGEN AND ITS GENE, Masahiko Tsuda, Kunihiko Yamaki, *Kuyas Bugra, *James T. Whelan, *James F. McGinnis and Toshimichi Shinohara, Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD 20892., *UCLA Schol of Medicine, Los Angeles, CA 90024.

S-antigen (S-Ag) is a well characterized highly antigenic retinal and pineal gland protein responsible for the induction of experimental autoimmune uveitis in experimental animals. Also, it has an important role in phototransduction. In order to understand the physiological function and the antigenic structure of this protien, it is essential to first know the structure of S-Ag.

We have previously characterized bovine retinal S-Ag cDNAs, the primary and secondary structure of the polypeptide, and immunogenic sites. Here, we present the primary structure of S-Ag polypeptide and its gene isolated from the inbred mouse (BALB/c). Mice total eyeball library was constructed by the method of Gubler and Hoffman. cDNA corresponding to S-Ag was isolated by cross hybridization with bovine probe and sequenced. Polypeptide sequence of the mouse S-Ag deduced from DNA sequence revealed extensive similarity with bovine sequence but interestingly, some regions have much difference, including c-terminus and middle of polypeptide. The uveitopathogenic sites of bovine and mosue have the same sequence. Putative phosphoryl binding sites and rhodopsin binding site in bovine were also conserved in mice.

Mouse gene fragments corresponding to one half of 3'-end of S-Ag were isolated and sequenced. Southern Blot hybridization analysis indicated S-Ag gene was a single copy. The splicing junctions of the gene were in good agreement with the consensus sequence, and the gene has long stretches of introns and very short exons.

I 107 γ-SUBUNIT OF MOUSE RETINAL cGMP-PDE: cDNA SEQUENCE AND mRNAs IN NORMAL AND rd RETINAS. Narendra Tuteja and Debora B. Farber, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024-1771.

Retinal cyclic GMP-phosphodiesterase (cGMP-PDE) is a key enzyme in phototransduction of rod photoreceptors; it has been shown to be involved in the normal functioning of these cells. A deficiency in cGMP-PDE activity causes an accumulation of cGMP in visual cells of rd mouse retina which results in their degeneration. Bovine cGMP-PDE has three subunits α, β and γ, MW 88, 84 and 11 Kd, respectively. The γ subunit contains 87 amino acids and the corresponding cDNA has 833 nucleotides (including coding and untranslated regions). We have cloned and sequenced a cGMP-PDEγ cDNA from mouse retina. A cDNA library was prepared in λgt10 from total retinal RNA isolated from normal C57BL/6N mice. The library was screened for cGMP-PDE$_\gamma$ cDNA using an oligonucleotide (30 mer) probe which we synthesized based on the published nucleotide sequence of bovine cGMP-PDE (Ovchinnikov Yu.A., et al. FEBS Letters 204:288, 1986). One positive clone of about 500 bp was isolated. Our preliminary data of nucleotide sequence and corresponding amino acid sequence of this cDNA shows that the coding region has very high homology with bovine cGMP-PDE$_\gamma$, with some nucleotides substituted but only a few amino acids changed. When transblots of retinal RNA from normal and rd 10-day-old mice were hybridized with the ^{32}P-labeled cGMP-PDE$_\gamma$ cDNA, a single band of about 900 bp was detected in both samples. This indicates that the mRNA coding for cGMP-PDE$_\gamma$ in the rd retina is probably similar to that in normal retina and suggests that if the defect in the rd disease resides in the cGMP-PDE molecule, it may be localized in its α or β subunits.
Supported by the National Retinitis Pigmentosa Foundation, Baltimore.

I 108 STRUCTURAL ANALYSIS OF HUMAN S-ANTIGEN AND ITS GENE, Kunihiko Yamaki and Toshimichi Shinohara, Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD 20892.

Bovine S-Antigen (S-Ag) is a well characterized highly antigenic protein present in retina and pineal gland. This protein is responsible for induction of experimental autoimmune uveitis (EAU). Also, S-Ag plays an important role in phototransduction. Previously, we have characterized bovine S-Ag and its cDNA. Here, we present the human S-Ag and its gene.

Several human S-Ag cDNAs were obtained from human retinal cDNA library (from J. Nathans) by hybridization with bovine S-Ag 5' end probe (340 base pair). Eight of them contained the full coding sequence and had the same restriction sites for Bgl II, Eco RI, Kpn I, Sph I, Sac I and Xho I. Interestingly, Hae III sites in the coding sequence varied suggesting that S-Ag may be polymorphic since the cDNA library was constructed from multiple retinas. The complete amino acid sequence of human retinal S-Ag has been determined by cDNA sequencing. A 1587 bp cDNA contains an open reading frame coding for a protein of 404 amino acids and 45,095 daltons. No extensive sequence homology was found between S-antigen and other proteins. However, local regions of sequence similarity with α-transducin are apparent including the pertussis toxin ADP-ribosylation site and phosphoryl binding sites. In addition, human S-antigen has sequences identical to bovine uveitogenic sites, indicating that some types of human uveitis may in part be related to the animal model of EAU. Also, we isolated and characterized human S-Ag gene. It was approximately 50 kilo base pair long and 3~4% was exon and 96~97% was intron.

Gene Expression and Differentiation

I 200 LIGHT/DARK CYCLING OF OPSIN, G-PROTEIN AND 48-kDa PROTEIN mRNAs IN THE DEVELOPING DEGENERATIVE rd AND ADULT NORMAL MOUSE RETINA. Cathy Bowes*# and Debora B. Farber*
*Jules Stein Eye Institute and #Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024.

Retinal degeneration in rd mice is manifested during the most rapid period of postnatal differentiation and is hypothesized to be caused by a lesion in cGMP metabolism. We have previously described the sequence of developmental expression of the mRNAs coding for three proteins involved in the cGMP cascade (opsin, G-protein and 48-kDa protein) in rd and control mouse retinas (IOVS Suppl 28, 345, 1987). Here, we have investigated whether there is a daily rhythm of mRNA synthesis of these proteins in developing rd/rd and rd/+ and in adult normal (+/+) mouse retinas. We find that the levels of each mRNA analysed do appear to cycle in the +/+ adult mouse retina, with the greatest amount of opsin and the three subunits of G-protein mRNAs occuring just before light onset and the greatest amount of 48-kDa protein mRNA occuring just before lights off. Cycling in the developing diseased or control retinas (P0-P12), however, is not observed and may be masked by the pronounced cell growth that occurs during this period.

Supported by the National Retinitis Pigmentosa Foundation Fighting Blindness, Baltimore.

I 201 THE SEQUENCE HOMOLOGY OF VERTEBRATE GAMMA-CRYSTALLINS AND HEAT-SHOCK PROTEIN GENES AND THEIR CORRESPONDING POLYPEPTIDES, S.-H. Chiou, T. Chang and W.-C. Chang, Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, P.O. Box 23-106, Taipei, Taiwan, ROC.

A systematic characterization of lens crystallins from five major classes of vertebrates was carried out by exclusion gel filtration, cation-exchange chromatography and N-terminal sequence determination. All crystallin fractions except that of gamma-crystallin were found to be N-terminally blocked. Gamma-crystallin is present in major classes of vertebrates except the bird, which showed none or decreased amounts of this protein in the chicken and duck lenses respectively. N-terminal sequence analysis of the purified gamma-crystallin polypeptides showed extensive homology between different classes of vertebrates, supporting the close relatedness of this family of crystallin even from the evolutionarily distant species. A comparison of the nucleotide sequences and their predicted amino acid sequences between gamma-crystallins of carp and rat lenses and heat-shock proteins revealed partial sequence homology of the encoded polypeptides and striking homology at the gene level. The unexpected strong homology of cDNA lies in the regions coding for 40 N-terminal residues of carp gamma-II, rat gamma2-1 and the middle segments of 23- and 70-kDa heat-shock proteins. The optimal alignment of DNA sequences along these two segments shows about 50% homology. The percentage of protein sequence identity for the corresponding aligned segments is only 20%. The weak sequence homology at the protein level is also found between the invertebrate squid crystallin and rat gamma-crystallin polypeptides. These results pointed to the possibility of unifying 3 major classes of vertebrate crystallins into one alpha/beta/gamma superfamily and corroborated the previous supposition that the existing crystallins in the animal kingdom are probably mutually interrelated and share a common ancestry.

I 202 EXTRA-LENTICULAR CRYSTALLIN TRANSCRIPTION AND EXPRESSION IN VIVO AND IN VITRO: EFFECT OF TISSUE OF ORIGIN, GENOTYPE AND GROWTH REGULATING FACTORS, RM Clayton, MW Head, J Cuthbert, A Peter & R Morrison, Dept. Genetics, University of Edinburgh, Edinburgh EH9 3JN, U.K.

Several crystallin genes are transcribed in chick neural retina and pigment ephelium, between 6.5 days of incubation and hatching. The cell population is heterogeneous with respect to a given crystallin but many cells appear to transcribe more than one crystallin. Levels of transcription and of crystallin proteins change in vivo or in vitro in temporal sequences and at quantitative levels which are tissue-related. Expression is modified by the genotype, and by several agencies affecting cell growth and differentiation, including cellular factors. The properties of the cell factors, and the cellular responses to them, both vary genetically. Each agency tested affects crystallins differentially and expression of a specific crystallin may be modified by several different agencies.

I 203 MACROPHAGE-MEDIATED OCULAR DISEASE IS AN EARLY DEVELOPMENTAL DEFECT IN TRANSGENIC MICE BEARING A HYBRID HEMOPOIETIC GROWTH FACTOR GENE. R Andrew CuthbertsonΔ, R Lang+ A Dunn+, JD PenschowΔ, I LyonsΔ, GK Klintworth*, D Metcalf° and JP CoghlanΔ. ΔHoward Florey Institute of Experimental Physiology and Medicine, University of Melbourne, 3052, Australia; +Ludwig Institute for Cancer Research and °Walter and Eliza Hall Institute, PO RMH, 3050, Australia; *Duke Eye Center, Durham, NC 27710.

We made transgenic mice that develop eye disease with the murine granulocyte-macrophage colony stimulating factor (GM-CSF) structural gene driven by the Molony virus promoter. Ocular disease, characterized by retinal destruction, cataractogenesis, corneal opacity and vitreous neovascularization, was apparent when affected animals opened their eyes 14 days after birth. The disease was mediated by destructive intraocular cells which were phagocytic and stained with monoclonal antibodies F4/80 and anti-Mac1, which with their morphology suggested that they were macrophages.
Hybridization histochemistry, using ^{32}P-labeled single strand RNA probes to GM-CSF and to a transcribed ØX174 bacteriophage DNA marker in the transgene construct, showed that transgene expression was in the intraocular macrophages themselves and that ocular disease might be caused by macrophage autocrine stimulation. Transgene expression was modulated to a peak between 14 and 21 days after birth, corresponding to the period of maximal tissue destruction.
In this model, expression of the introduced gene during ocular development may mediate autocrine stimulation of intraocular macrophages resulting in severe ocular pathology.

I 204 CIS-ACTING SEQUENCES REQUIRED FOR EXPRESSION OF THE αB-CRYSTALLIN GENE. R. Dubin and J. Piatigorsky. Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

The crystallins compose several families of proteins abundantly expressed in the vertebrate lens. The α-crystallins are encoded by two genes, αA and αB. Previous work in this laboratory has demonstrated that sequences between -111 and +46 of the mouse αA gene are sufficient to confer lens specific expression of a reporter gene in embryonic chicken lens explants and transgenic mice. Additionally, two separable domains have been functionally identified within this region and both bind chick lens nuclear proteins.

To extend our understanding of the regulated expression of the α-crystallin genes, we have isolated the complete mouse αB gene and the 5' portion of the human αB gene. A mouse αB mini-gene was constructed which contains 600 bp 5' to the transcription initiation site, parts of exons 1 and 3, and 2.5 kb downstream from the poly A addition site. Following transfection this gene is transcribed in embryonic chick lens epithelial cells. To further define transcriptional regulatory regions, 5' and 3' deletion mutants of the αB mini-gene have been generated and their ability to direct accurate tissue specific transcription is in progress. The mouse and human αB promoters have also been linked to the bacterial CAT gene in order to determine whether 5' sequences are sufficient for tissue specific expression and if so, whether conserved sequences are involved.

I 205 DEVELOPMENTAL REGULATION OF A MURINE γ-CRYSTALLIN AND BACTERIAL LACZ FUSION GENE IN TRANSGENIC MICE. Daphne R. Goring[1,2], Janet Rossant[1,3], Susan Clapoff[3], Martin L. Breitman[1,3] and Lap-Chee Tsui[1,2]. [1]Department of Medical Gentics, University of Toronto, Ontario M5S 1A8; [2]Genetics Department, Hospital for Sick Children, Toronto, Ontario M5G 1X8; and [3]Mount Sinai Hospital Research Institute, Toronto, Ontario M5G 1X5, Canada.

Expression of the γ-crystallin gene family is restricted to the terminally differentiated fibre cells of the mammalian lens. Individual γ-crystallin genes are differentially regulated during development, resulting in a precise spatial distribution of γ-crystallins across the lens. We have shown that a hybrid gene containing 759 basepairs (bp) of the murine γ2-crystallin promoter fused to the coding region of the *E. coli lacZ* gene was expressed in transgenic mice in a tissue-specific manner. The *in situ* β-galactosidase (β-gal) assay performed on eye sections from 6 week-old mice revealed that the enzyme activity is restricted to the nuclear lens fibre cells and not in the lens epithelial cells. This result is thus in general agreement with the temporal and spatial regulation of the endogenous γ2-crystallin gene. To further characterize the expression of the transgenes, enzyme activity and RNA levels were examined at various stages of lens development. In all three transgenic lines, β-gal RNA and enzyme activity could be detected in 16 day-old embryos, corresponding well with the initiation of the endogenous γ2-crystallin gene expression. However, careful examination of the β-gal activity across the lens and RNA levels at different ages showed that the distribution of β-gal containing fibre cells in the 3 transgenic lines is significantly different from each other. This variation is probably due to the effect of adjacent chromosomal sequences, resulting in different durations of transgene expression. Moreover, our preliminary data indicated that 226 bp of the γ2-crystallin promoter is sufficient to direct tissue-specific as well as developmental regulation of the transgene.

I 206 A SINGLE COPY GENE IN DUCK ENCODES BOTH ε-CRYSTALLIN AND LACTATE DEHYDROGENASE-B,
Wiljan Hendriks, John W.M. Mulders, Hans Bloemendal and Wilfried W. de Jong, University of Nijmegen, Nijmegen, The Netherlands.
Water soluble proteins that occur in large amounts in vertebrate lenses are called crystallins. A few years ago an additional major lens protein, described as ε-crystallin, was found in many bird and crocodile lenses. Recently, it was shown that ε-crystallin is an active glycolytic enzyme, lactate dehydrogenase (LDH), and that duck ε-crystallin appears to be identical to duck LDH-B4 (Wistow, G. et al. (1987) Nature **326**, 622-624). To answer the question whether the abundant lens LDH/ε-crystallin is the same gene product as authentic heart LDH-B4, or that the two proteins are rather the products of very similar, recently duplicated genes, we now have isolated and sequenced an almost full-length cDNA clone of duck ε-crystallin. Using this clone as a probe we demonstrate, by restriction enzyme mapping and Southern blotting, that there is a single copy Ldh-b gene in chicken and in duck. This Ldh-b gene is overexpressed in the eye lenses of duck, but not in chicken. Active LDH-B4 thus makes up some 10% of the duck lens protein, and differs from the primary gene product probably as a result of post-translational deamidation at positions 163 and 265.

I 207 A TRANSCRIPTIONAL REGULATORY REGION IN THE 5' FLANKING SEQUENCE OF THE CHICKEN αA-CRYSTALLIN GENE. John F. Klement and Joram Piatigorsky. Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

To delineate the chicken αA-crystallin 5' flanking region required for transcription, we fused various lengths of upstream DNA sequence, along with 72 bp of the first exon, to the bacterial chloramphenicol acetyltransferase (CAT) gene and transfected 14-day old chicken lens epithelial cells or fibroblasts. Our results indicated that 163 bp of αA-crystallin 5' DNA flanking sequence were required for efficient and tissue-specific expression of the αA-crystallin/CAT gene. The αA-crystallin 5' flanking region from -163 to -122 was protected from exonuclease digestion in the presence of lens nuclear extract from 14-day old chicken embryos. The 5' promoter deletion and DNA protection experiments suggest that the -163 to -122 region of the chicken αA-crystallin gene contains a binding site(s) for a transcription activating factor(s). Okazaki et al. (EMBO J. 4, 2589-2595, 1985) have reported that the chicken αA-crystallin 5' flanking region from -242 to -189 was required for the efficient expression of a chicken αA/δ-crystallin chimeric gene microinjected into mouse lens cells. Chepelinsky et al. (Mol. Cell Biol. 7, 1807-1814, 1987) demonstrated that 111 bp of 5' flanking sequence of the homologous mouse αA-crystallin gene was sufficient for the expression of an αA-crystallin/CAT gene in embryonic chicken lens epithelia. Together the data suggest that the regulation of the αA-crystallin gene in mice and chickens may involve species specific factors or different arrangements of common regulatory regions.

I 208 ß1-TUBULIN GENE EXPRESSION IS RELATED TO THE OPTIC NERVE OUTGROWTH DURING CHICK EMBRYO DEVELOPMENT. J.-M. Matter, L. Matter-Sadzinski, I. Bakst and W.M. Cowan, Washington University, School of Medicine, St Louis, MO 63110.
The expression of the ß1-tubulin gene is stimulated during a short period of the chick retina development. The expression of this gene is restricted to the ganglion cell population and it coincides with the growth of the optic nerve axons. The transient expression of the ß1-tubulin gene is quantitatively similar in the retinal ganglion cells and in other neurons of the CNS which are developing their axons, and we have found that expression of this gene may be used as a marker of the axonal outgrowth. The stimulation of expression of the ß1-tubulin gene in the ganglion cells is independent on the stage of development of the embryonic tissue surrounding the eye; in contrast, it depends on the stage of development of the eye, and our results suggest that the onset of the optic nerve outgrowth is autonomously regulated by the eye itself. In vitro, the ß1-tubulin gene is expressed in retina explants only when the culture conditions promote axon outgrowth. We have developed an in vitro assay which enables us to identify in the retina explant the ganglion cells which are actively extending their axons and to analyse the gene expression exclusively in this cell population.

I 209 TWO PHENOTYPICALLY DISTINCT LENS TUMORS IN TRANSGENIC MICE BEARING an αA-CRYSTALLIN-SV40-T ANTIGEN TRANSGENE, Takafumi Nakamura, Kathleen Mahon, Anup Dey and Heiner Westphal, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, MD 20892.

Transgenic mice bearing a chimeric gene consisting of the 5' regulatory sequence from the αA-crystallin gene fused to the coding region of the SV40 T antigens (Tag) develop heritable lens tumors (Science 235, 1622-1628, 1987). Mice from line αTag-1 develop poorly differentiated carcinomas of the lens epithelium which become vascularized and grow to fill the eye cavity by 2-3 months of age. Line αTag-1 mice are of poor health and die at 3-4 months of age. In contrast, mice from the αTag-2 line are vigorous and healthy and develop histologically distinct, moderately well differentiated carcinomas of the lens epithelium which do not fill the eye cavity until ~1 year of age. Line αTag-2 lens cells produce higher levels of all three classes of lens crystallins (α,β,γ) than αTag-1 lens cells. However, αTag-1 and αTag-2 mice are indistinguishable in lens tumor transplantability and in their immunological tolerance for Tag. Lens cells from both lines synthesize comparable levels of small and large Tags, suggesting that Tag alone is not sufficient to bring about the observed difference in malignancy between αTag-1 and αTag-2 mice. In addition, mice from both lines develop secondary non-lens tumors in several organs. The tumors of αTag-1 mice grow more rapidly than those of αTag-2. The secondary tumors of αTag-1 mice produce higher levels of large and small Tags than the lens tumors, but do not produce lens crystallins. DNA analysis of these secondary tumors show neither amplification nor rearrangement of integrated genes.

I 210 βB1-CRYSTALLIN GENE EXPRESSION: EVIDENCE FOR AT LEAST TWO PROMOTER REGULATORY ELEMENTS. H. John Roth and Joram Piatigorsky, National Eye Institute, NIH, Bethesda, MD 20892.

The β-crystallins of the chicken eye lens are composed of seven different polypeptides (19 kDa to 35 kDa). The genes encoding these β-crystallin polypeptides each exhibit a characteristic temporal and spatial pattern of expression in the developing lens. The βB1-crystallin polypeptide is unique since mRNA encoding this polypeptide accumulates only in the equatorial epithelial and fiber cells, but not in the central epithelial cells, of the chicken lens. To identify sequence elements of the chicken βB1-crystallin gene responsible for regulating its expression, we have monitored the ability of cloned 5' flanking gene sequences introduced into the pSVO-CAT expression vector to direct chloramphenicol acetyltransferase (CAT) gene expression in cultured cells. Co-transfection with an RSV-β-Gal expression vector provided an internal control and allowed normalization of the results. Three patterns of CAT expression were observed depending on the number of 5' flanking basepairs (bp) fused to the CAT gene: 500 bp showed moderate activity in chicken lens cells but no activity in chicken fibroblasts, 294 and 149 bp showed high activity in lens cells and moderate activity in fibroblasts, and 98 bp showed negligible activity in both lens cells and fibroblasts. These data indicate the presence of at least two distinct regulatory elements in the promoter region of the chicken βB1-crystallin gene: a lens-specific element between -500 and -295 bp, and a general transcriptional activating element between -149 and -99 bp.

I 211 PHOTORECEPTOR DEGENERATION LEADS TO GFAP GENE EXPRESSION IN THE MOUSE RETINA, P. Vijay Sarthy and Marlene Fu, Department of Ophthalmology, University of Washington, Seattle, WA 98195.

Glial fibrillary acidic protein (GFAP) is a 50 KDa protein that is the major constituent of glial intermediate filaments found in astrocytes. In normal mouse retina, GFAP-antisera stain only the astrocytes while Müller cells, the predominant glial cells, remain unstained. Photoreceptor degeneration resulting from retinal degeneration (rd), nervous (nr) mutations, or constant environment light damage leads to the appearance of GFAP-staining in Müller cells. We have investigated the mechanism behind this phenomenon. Western blot analysis showed that there was 5 to 10-fold increase in GFAP levels in retinas with photoreceptor degeneration. In Northern blots, a 10 to 25-fold increase in GFAP mRNA was found in these retinas. Finally, in situ hybridizations with sections of retina as well as dissociated Müller cells clearly established that GFAP mRNA levels were elevated in Müller cells. These results show that photoreceptor degeneration leads to induction of the GFAP gene in Müller cells of the mouse retina. (Supported by NIH Grants EY03664, EY03523, and EY01730.)

I 212 INCREASES IN CALPACTIN I mRNA LEVELS DURING THE DIFFERENTIATION OF EMBRYONIC CHICKEN LENS EPITHELIAL CELLS INTO LENS FIBER CELLS. J. Talian and P. Zelenka, National Eye Institute, NIH, Bethesda, MD 20892

Calpactin I is a calcium-dependent phospholipid and actin-binding protein which is a known substrate of the pp60^{src} kinase. It is immunologically identical to the major component of the EDTA-extractable protein fraction (EEP) of lens membranes. Northern blot analysis of chicken lens fiber cell RNA with a bovine calpactin I cDNA probe reveals the presence of a 1.8 Kb calpactin mRNA in these cells. To investigate the calpactin I mRNA levels during differentiation we used an in vitro system consisting of explants of central lens epithelia. These explants are devoid of fiber cells but can be stimulated to differentiate by addition of IGF-like compounds. Northern blots of RNA samples prepared at different times following initiation of differentiation indicate a 2-5 fold increase in calpactin mRNA during the differentiation process. We have also examined differentiating explants and histological sections of whole lenses with an antibody directed against calpactin I. Immunofluorescence demonstrates the presence of calpactin I in the basal region of epithelial cells adjacent to the lens capsule and in the punctate distribution near the plasma membrane of elongating fiber cells. The increased mRNA levels and the immunological localization of the protein suggest a possible role of calpactin I in the morphological alterations which accompany lens cell differentiation.

I 213 MOLECULAR ANALYSIS OF GENES RESPONSIBLE FOR RETINAL DEGENERATION, Gabriel H. Travis and J. Gregor Sutcliffe, Research Institute of Scripps Clinic, La Jolla, CA 92037

Four mouse mutants have been described in which the retina develops normally but subsequently undergoes degeneration with complete or near-complete loss of photoreceptor cells: retinal degeneration (rd), retinal degeneration slow (rds), Purkinje cell degeneration (pcd) and nervous (nr). Each of the four mutations display autosomal recessive inheritance and the chromosomal location of each mutation is known, however the underlying molecular etiology is not understood. At least two of these mutations (rd and pcd) act in a cell-autonomous manner, demonstrated by analysis of experimental chimaeras. In an effort to understand the process of neuronal degeneration and to gain further insight into photoreceptor cell physiology, we are attempting to clone the mouse genes responsible for each of these mutations. Our strategy is based upon a new subtractive cDNA cloning procedure that requires only small quantities of poly(A)$^+$ RNA and permits the isolation of cDNA clones representing differentially expressed mRNAs of abundances as low as 0.001%. Subtractive hybridization of normal retina cDNA against rd mutant retina was performed in order to isolate a group of rod cell-specific mRNAs. The chromosomal assignment of the gene encoding each mRNA is being made by Southern blot analysis of DNA from mouse-hamster hybrid cell lines. Rod cell-specific mRNAs that map to the appropriate mouse chromosomes represent candidate transcripts of the mutant genes. These clones will be analyzed by Northern blot analysis of RNA from the retina of both normal and mutant animals taken prior to cellular degeneration, by ribonuclease-protection analysis of normal and mutant RNA, and by Southern blot analysis of normal and mutant mouse DNA.

I 214 DIFFERENTIAL EXPRESSION OF THE N-RAS PROTO-ONCOGENE IN METAPLASTIC RETINAL PIGMENTED EPITHELIUM, David K. Wilcox and Jean-Paul Vergnes, The Eye and Ear Institute of Pittsburgh, Pittsburgh, PA 15213

Retinal pigmented epithelial (RPE) cells incubated in the presence of vitreous undergo a metaplasia from an epithelial to a fibrocytic morphology. We studied the levels of n-ras RNA in normal and metaplastic RPE. RPE cells were incubated for 24 hours in DMEM:Hams F-12 ("basal media") containing only 0.5% BSA. These "starved" cells were then stimulated with either normal media (basal media supplemented with insulin, transferrin, selenium, and 5% fetal calf serum) or basal media supplemented 1:1 with vitreous and 0.5% BSA. Stimulation with both normal and vitreous supplemented media resulted in similar levels of DNA synthesis. At 24 hours post-stimulation TCA precipitable H3-thymidine incorporation increased over two fold to 1.8 and 1.9 x 10-4 cpm/10-5 cells in normal and vitreous supplemented media, respectively. In cells stimulated with normal media a decrease in n-ras RNA levels was apparent within 30 minutes with an almost total loss of detectable n-ras RNA at 60 minutes post-stimulation. This was followed by an increase of ~ 10-fold by 6 hours post-stimulation. By 18 hours cells stimulated with normal media had returned to pre-stimulation levels. In contrast, the vitreous stimulated cells retained pre-stimulation levels of n-ras RNA and by 6 hours post-stimulation RNA levels had increased ~ 20-fold. These levels decreased slowly and remained 2-4 fold above control levels at 18 hours post-stimulation. Levels of another proto-oncogene,neu, remained unaltered. Elevated levels of n-ras may play a role in the RPE metaplasia associated with pathologies such as proliferative retinopathy. (Supported by NEI EY06479)

I 215 INCREASE IN TUBULIN MRNA IN THE FROG RETINA DURING OPTIC NERVE REGENERATION, Y.Yagi*,T.Mizobuchi,A.Mizuno,H.Matsuzaki and M.Matsuda,Jikei University,Tokyo 105.

In lower vertebrates, such as frogs or fish, the crushed nerve fibers of the central nervous system can elongate their axons and recover their functions.

The optic nerve of the bull frog (Rana catesbeiana) was transected and the process of the regeneration was investigated. Three months after the operation, the microscopic examination showed no atrophic change of the optic nerve fibers. The regenerated nerve bundles appeared to be normal except for some irregularities at the site of transection. The proteins labelled with (3H) leucine in the retina were transported to the tectum and the amount of their radioactivity was roughly the same as that of those in the retina in a normal, unoperated frog. Following the transection of optic nerve, a rapid and transient increase in messenger RNA of the tubulin gene was observed in the retina, but no specific changes in actin messenger RNA were observed. The rapid expression of the tubulin gene suggests that the optic nerve transection causes an immediate response in the transcription level of the tubulin gene in a retinal ganglion cell.

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Ocular Disease

I 300 ANALYSIS OF ANTIGENIC DETERMINANTS OF RETINAL S-ANTIGEN WITH MONOCLONAL ANTIBODIES, J Paul Banga, Eva Kasp and Dudley Dumonde, Dept. of Medicine, Kings College Hospital and Department of Immunology, St Thomas' Hopsital, London, U.K.

Eight S-antigen specific monoclonal antibodies have been used to map the antigenic determinants of this retinal autoantigen. By competitive binding analysis, three antigenic clusters of bovine S-antigen were recognised to which monoclonal and polyclonal antibodies are directed. Immunohistochemistry on retinal sections from eyes of several mammalian species reconfirmed the presence of three different epitopes on S-antigen. In addition, some heterogeneity in the staining pattern of some of the monoclonal antibodies was evident on bovine retinal sections. By Western blotting on cyanogen bromide derived peptide fragments of bovine S-antigen, two of the antigenic clusters recognised by the monoclonal antibodies were localised to epitopes contained on two peptide fragments of 26,000 and 18,000 molecular weight. The same peptide fragments also react with antiserum to bovine S-antigen raised in rabbits and rats. Monoclonal antibodies to the third antigenic cluster do not show any reactivity by immunoblotting on intact or peptides of S-antigen. These observations suggest that S-antigen contains both linear and topographic antigenic determinants.

I 301 X-LINKED PROGRESSIVE CONE DYSTROPHY - CLINICAL CHARACTERISTICS AND LINKAGE TO POLYMORPHIC DNA PROBES, J. Bartley, C. Gies and D. Jacobsen*, Department of Pediatrics and Ophthalmology*, University of Iowa Hospitals, Iowa City, IA 52242.

A large family spanning four generations with a progressive visual loss was evaluated with a neurological and a neuro-ophthalmological examination. Nine affected males, three unaffected males, five obligate carrier females and four unaffected females were examined. By the fifth decade a plateau of visual deterioration was reached which included daylight glare and photophobia, and visual acuity of 20/100. Older patients were unable to negotiate unassisted and had visual acuities as poor as 20/800 to counting fingers at three feet. Patients younger than 30 years had generalized depression of the field of vision (Goldmann perimetry) while older patients had central scotoma. All patients had markedly defective color vision. All patients in the first three decades of life had maculas that appeared darker and more grandular than usual; the fovea was indistinct or not identified within the macula. The retinal pigment epithelium (RPE) was diffusely thin. Patients in their seventh through ninth decades had well demarcated regions in the macula of almost complete loss of RPE. Photopic ERG B wave amplitudes were greater than 2 S.D. below the mean in all patients. The oldest and most severely affected patient had absent ERG responses. No patient had neurological symptoms, other than those of vision abnormalities. All female obligate carriers were normal. DNA prepared from lympocytes of members of this family has been digested with restriction enzymes, electrophoresed on agarose gels, transferred by Southern blotting to nylon filters and hybridized with ^{32}P-labelled random X-linked probes. Preliminary data shows no recombinations occurred between Xp21 probes and this disorder.

I 302 RECOMBINATIONAL EVENT BETWEEN NORRIE DISEASE AND DXS7 LOCI.
J. Bronwyn Bateman, Julielani T. Ngo, M. Anne Spence, Victoria Cortessis, and Robert S. Sparkes. From the Jules Stein Eye Institute and Departments of Ophthalmology, Psychiatry, Biomathematics, Medicine and Pediatrics, UCLA School of Medicine, Los Angeles, California.

We have identified a family affected with X-linked recessive Norrie disease in which a recombinational event occurred between the disease locus and the DXS7 locus identified by the probe L1.28. The addition of our family brings the total of published informative families to seven with a maximum lod score of 7.58 at a recombination frequency of 0.038 ± 0.036. This finding indicates that the L1.28 probe is useful but may not be completely reliable for prenatal diagnosis and that the gene for Norrie disease is not within the DNA sequence identified by the L1.28 probe.

I 303 ANALYSIS OF HSV ACTIVITY IN A DRUG-SUPPRESSION MODEL OF HSV INFECTION.
KM Bean, EC Dunkel and D Pavan-Langston.
Eye Research Institute and Harvard Medical School, Boston, MA, 02030

Trigeminal ganglia were removed from 3 to 4 day old NZW rabbits, plated directly onto $35mm^2$ culture dishes and rehydrated with MEM containing nerve growth factor and incubated at 37C for three days (neurite extension 3-5mm).

Established trigeminal ganglion neuron cultures were inoculated with 100ul of 10^5 PFU McKrae strain HSV-1 by adsorbtion for 1 hour at 37C. Following adsorption, the cultures were hydrated with MEM containing 100ug/ml acyclovir. When infectious virus could no longer be recovered from the cultures by supernate or cell-free homogenate culture onto Vero cells, the HSV infection in the neuron cultures was considered to be suppressed.

Acute and drug-suppressed neuron cultures were harvested on days 3 and 6 post-suppression by centrifugation in alcohol and processed for in situ nucleic acid hybridization with a full-length HSV DNA cloned probe.

Following in situ hybridization, trigeminal ganglion neurons selected during acute infection (day 3 post-suppression) and suppressed infection (day 6 post-suppression) both reacted positively to the probe with acutely infected cultures demonstrating a more intense hybridization reaction and greater numbers of reacting neurons. An analysis of the contribution of HSV RNA present in this neuronal drug-suppression model will be presented.

I 304 HUMAN RETINOBLASTOMA SUSCEPTIBILITY GENE: GENOMIC ORGANIZATION AND ANALYSIS OF HETEROZYGOUS INTRAGENIC DELETION MUTANTS, Robert Bookstein, Eva Y.-H. P. Lee, Hoang To, Lih-Jiuan Young, and Wen-Hwa Lee, Department of Pathology M-012, University of California, San Diego, La Jolla, CA 92093. A gene in chromosome region 13q14 has been identified as the human retinoblastoma susceptibility (RB) gene on the basis of altered gene expression found in virtually all retinoblastomas. Complete or partial deletions of the RB gene have been observed in about 40% of such tumors. In order to further characterize the RB gene and its structural alterations, we examined genomic clones of the RB gene isolated from both a normal human genomic library and a library made from DNA of retinoblastoma cell line Y79. First, a restriction and exon map of the RB gene was constructed by aligning overlapping genomic clones, yielding three contiguous regions (contigs) of 150 kilobases (kb) total length separated by two gaps. At least eighteen exons were identified in genomic clones, and these were provisionally numbered. Second, two overlapping genomic clones were isolated from the Y79 library that demonstrated a DNA deletion removing exons 2 through 5 from one RB allele. To confirm and extend this result, a unique sequence probe pN6R0.5 in intron 1 was used to detect identical heterozygous deletions in genomic DNA from three retinoblastoma cell lines, Y79, RB355 and WERI-27, thereby explaining the origins of their shortened RB mRNA transcripts. The unexpected coincidence of deletion junctions indicates the presence of a mutational "hot spot" at this site. Third, probe pN6R0.5 also detected a polymorphic site for *Bam*HI with allele frequencies near 0.5 / 0.5. Use of this probe may thus contribute significantly to genetic diagnosis in retinoblastoma patients and families.

I 305 HERPES SIMPLEX VIRUS GENOMIC DIFFERENCES AND OCULAR DISEASE. Ysolina M. Centifanto, Tulane University School of Medicine, New Orleans, LA 70112.

We have previously shown that herpes simplex virus strains that infect the eye differ both in their disease manifestations as well as their reactivation patterns.

Restriction endonuclease analysis of the DNA of HSV isolates has been useful in the characterization of variant progeny within the population of a non-virulent strain. DNA digestion profile of this varient isolate showed that the genomic differences map within the terminal repeat of the unique long segment and the internal joint region, specifically in bands B, E, N, and S from a Bam HI digestion and bands M and N from a Hind III digestion profile.

In contrast, digestion profiles of HSV isolates from recurrent lesion or asymptomatic shedding of animals infected with a wild type strain, failed to show clear cut differences when analyzed by the same restriction endonuclease enzymes. In this case, the formation of a lesion or the asymptomatic shedding was determined by the immunological environment at the peripheral site (cornea).

I 306 EFFECT OF GINKGO BILOBA (EGB 761) TREATMENT IN ELECTROPHYSIOLOGY OF ALLOXAN INDUCED DIABETIC RATS ISOLATED RETINAS, Michel Doly, Marie-Therese Droy-Lefaix, Marie Madeleine Ruchoux, Pierre Braquet, IPSEN-IHB Research Laboratories, 92350 Le Plessis Robinson, FRANCE

The authors established the functional impairment due to diabetic retinopathy by performing experiments on isolated retinas of alloxan-induced diabetic rats.

After one month's evolution of diabetes, the electroretinograms (ERG) obtained on isolated retinas has an amplitude on average 20 % lower than in the controls ; whereas after two months of diabetes, this decrease in amplitude is around 60 %.
In a second stage, the authors compare the E.R.G. obtained on the isolated retinas of two months diabetic animals, non treated or treated per os with Ginkgo biloba extract (100 mg/kg/d) : these ERG have a significantly greater amplitude ($p < 0.001$) in treated animals.

The result may be discussed in terms of the role of oxygenated-free radicals in diabetic retinopathy and also in terms of the free-radical scavenger property of Ginkgo biloba extract, which could participate in the preventive treatment of the visual functional impairment involved in diabetes.

I 307 PROTECTIVE EFFECT OF GINKGO BILOBA EXTRACT (EGB 761) ON LIGHT DAMAGE IN THE RAT RETINA, Marie-Therese Droy-Lefaix, Marie-Madeleine Ruchoux, Joël Guillemain, IHB/IPSEN Research Laboratories, 92350 Le Plessis Robinson, FRANCE

Method : 120 Sprague-Dawley rats (225-275 g) were randomly divided into ten groups of 12 animals each. Six groups were light exposed (group 1 : control 1 day, group 2 : control 4 days, group 3 : placebo 1 day, group 4 : placebo 4 days, group 5 : EGB 761 1 day, group 6 : EGB 761 4 days. Four groups were not light exposed : group 7 : placebo 1 day, group 8 : placebo 4 days, group 9 : EGB 761 1 day, group 10 : EGB 761 4 days. In a ventilated chamber, rats were exposed under cyclic light (12h light, 12h dark) of 3,100 lumens provided by a set of 3.120 cm long fluorescent lamps with reflectors. In all cases drugs were administered by gastric intubation at the doses of 100 mg/kg/po EGB 761 and placebo under a volume of 2 ml of water. After 1 or 4 days rats were sacrificed. Retina was fixed in Trump liquid for an ultrastructural study.
Results : On control and placebo groups, exposure to light induced important damage of the rat retina. Electron microscopic study showed marked thickness of the pigment epithelium, vesiculation and complete desintegration of the disc membranes of the outer segments, swelling of the inner segments, pyknosis and cells swelling of the outer nuclearlayer and outer plexiform layer, organites clumping of the inner nuclear layer cells. With EGB 761, only minor changes were observed.
Conclusion : This study confirmed light retinal degeneration exposure. Damage of the retina may be due to an increase of the hydroperoxides level. The improvements observed may be consequential to the effect of Ginkgo biloba extract as a free-radical scavenger.

I 308 MOLECULAR ANALYSIS OF THE CANDIDATE RETINOBLASTOMA GENE LOCUS, James M. Dunn, Andrew J. Becker, Robert A. Phillips and Brenda L. Gallie, Hospital for Sick Children Research institute, and University of Toronto, Canada, M5G1X8.

Human retinoblastoma (RB) is the most common form of intraocular tumor in early childhood. Approximately 60% of RB patients develop only one tumor originating from one retinal precursor cell. The other 40% carry a germline mutation which predisposes them to multiple RB tumors and places them at risk for other tumors later in life. Although RB predisposition is an autosomal dominant trait, the gene is recessive at the cellular level, and involves loss of gene function. Therefore an RB patient carrying a germline mutation will have a normal and a mutated RB locus, but the tumor will have both alleles mutated.
The RB locus has been genetically and physically mapped to chromosome 13q14. Recently Friend et. al. (Nature 323:643, 1986) cloned a candidate gene, 4.7R, from this region. In our laboratory Southern blot analysis showed genomic mutations in 12% of the RB tumors tested, most of which are total deletions of the locus. Northern blots confirmed the Southern data and identified only one new mutation. Using RNAse protection, which can detect single base pair changes, no new mutations have been detected in RB tumors. The tumors with known mutations have been confirmed and a new DNA polymorphism has been found. These data suggest that 4.7R may only be a close neighbour to the RB gene.

I 309 OCULAR DISEASES IN AIDS: POSSIBLE DIRECT INFECTION OF CELLS BY HIV?, Kamla Dutt , Diane Dorsett , M. Delmonte , D. M. Albert , and A. Srinivasan . Morehouse School of Medicine and Centers for Disease Control, Atlanta, GA. Kellogg Eye Center, Michigan. Mass Eye and Ear Infirmary, Boston, MA.
Acquired immunodeficiency syndrome is associated with multiple ocular abnormalities including Kaposi's sarcoma of the lid and conjunctiva, cotton-wool spots and retinopathy. The role of HIV infection in these complications is not clear. HIV has been isolated from tears and corneas of patients with AIDS. It has been shown that HIV can infect corneal epithelial and endothelial cells in vitro. In this study we have analyzed a number of cells derived from ocular tissues for their susceptibility to HIV infection. We have developed a specific, sensitive, and quick assay to assess the infection of cells by HIV. This assay exploits transactivation features of HIV-TAT gene products which can be detected by transfecting the indicator plasmid pLTR-CAT. Choroid fibroblasts, corneal fibroblasts, retinoblastoma cells and retinal pigment epithelial cells were evaluated for infectibility by HIV employing reverse transcriptase, antigen and transactivation function assays.

I 310 MOLECULAR EVALUATION OF VARICELLA ZOSTER VIRUS INFECTION IN RABBIT CORNEAL EPITHELIAL CELLS: AN IN VITRO MODEL
PA GEARY, KM BEAN, CA VASLET, DP LANGSTON, EC DUNKEL
Eye Research Institute, Boston, MA 02114

Rabbit corneal epithelial cell monolayers were inoculated with 10^3 PFU Oka strain VZV by adsorption for 1 hour. After addition of 50ug/ml acyclovir (ACV) to the selected culture medium, cultures were harvested on days 1-18 post inoculation (PI). One and 5ug DNA samples were spotted onto nitrocellulose paper and hybridized with a [^{32}P] labeled VZV DNA probe representing full-length VZV DNA.

In ACV-suppressed VZV-infected cultures, a transient CPE was noted in 17% of the cultures on days 3-4 PI. In 91% of the ACV-suppressed cultures, no cell-free virus was detected during post-supression (PS) days 4-9. After ACV removal (day 9 PS), 38% of the cultures VZV was reactivated within 4 days resulting in infectious virus recovery from both supernatant and cell-free co-cultures.

Dot-blot analysis of nucleic acids extracted from control, non-suppressed VZV-inoculated cultures demonstrated the presence of VZV DNA at a 10pg level on days 3-4 PI and at >100pg on days 5, 6 and 7 PI. In ACV-suppressed VZV-inoculated cultures, 10pg of VZV DNA was detected on days 2-4 PS and 1-3pg on days 5-9 PS. After removal of ACV from the culture medium on day 9 PS, 10-100pg of VZV DNA was detected (days 10-18 PS).

I 311 LYMPHOCYTE CELL LINES, SPECIFIC TO A SYNTHETIC PEPTIDE DERIVED FROM IRBP, ARE HIGHLY UVEITOGENIC, I. Gery, L. H. Hu, H. Sanui, T. M. Redmond, B. Wiggert, T. Kuwabara and G. J. Chader, National Eye Institute, NIH, Bethesda, MD 20892.
In another study reported in this Meeting (T. M. Redmond et al) we have shown that a synthetic peptide derived from IRBP produces in Lewis rats uveoretinitis and pinealitis. The peptide, designated R4, consists of 23 amino acids. The disease induced by R4 was adoptively transferred to naive recipients by $\geq 10^7$ sensitized lymphocytes from donors immunized with the peptide. Moreover, two R4-specific lymphocyte cell lines were established, using lymph nodes of an actively immunized rat and spleen of a recipient as the cell sources. The line cells, which carry the markers of helper/inducer T lymphocytes, produced disease in naive rats within 4-5 days following injection of as few as 2×10^5 cells. The disease induced by the line cells resembled that induced by active immunization with R4. The line cells proliferated vigorously in culture when stimulated with R4 or a closely related peptide, R9, while only low responses were stimulated by the whole IRBP molecule. No responses were stimulated by other non-related peptides derived from IRBP.

I 312 MOLECULAR ANALYSIS OF A REARRANGEMENT IN THE PUTATIVE RETINOBLASTOMA GENE, Audrey D. Goddard, Robert A. Phillips and Brenda L. Gallie, Dept. of Medical Biophysics, University of Toronto and Division of Hematology/Oncology, The Hospital For Sick Children, Toronto, Canada.
An initial screening of 32 Retinoblastoma (RB) and 2 Osteogenic Sarcoma (OS) tumors revealed a low frequency (12%) of deletion and/or rearrangement in the gene detected by the putative Rb1 cDNA clone 4.7R (Friend et al., Nature 1986;323:643-646). One tumor, RB369E, appears to have two independent rearrangements involving this gene: 1) a 3' deletion affecting the three 3' most HindIII fragments containing exons; and 2) a rearrangement in an intron near the 5' end of the gene, which is most likely an insertion of approximately 19kb. The latter rearrangement results in junction fragments with a number of restriction enzymes. Cloning and characterization of this rearrangement will provide insight into mutations in human genes and may provide evidence the 4.7R is the Rb1 gene.

I 313 ANTISENSE RNA TRANSCRIPTS TO HERPES SIMPLEX ICP-0 GENE ARE PRESENT IN HUMAN TRIGEMINAL GANGLIA, Y. Jerold Gordon, Bruce Johnson, Trinita Araullo-Cruz and Eric Romanowski, The Eye & Ear Institute, University of Pittsburgh, Pgh, PA 15213

Recent animal studies demonstrated abundant antisense RNA transcripts to the herpes alpha gene ICP-0 in latently-infected ganglia. We investigated the situation in human trigeminal ganglia (TG) removed from 14 unselected autopsied cases. Strand-specific 2.7 kb HSV-1 ICP-0 RNA probes were radiolabelled, and their specificity and sense established in HSV-1 productively-infected Vero cells. Using in situ hybridization, the ICP-0 RNA probes were hybridized to the TG sections, which were then washed, exposed to NTB-3 emulsion, developed, stained and counted. Antisense ICP-0 RNA transcripts were demonstrated in the nuclei of neurons in 46% of human ganglia, while true ICP-0 messenger RNA was NOT found in any of the ganglia. We conclude that HSV-1 antisense ICP-0 RNA is present in humans during ganglionic latency.

I 314 BLUE CONE MONOCHROMACY: ASSIGNMENT OF THE LOCUS TO Xq28, INITIAL CHARACTERIZATION OF THE MOLECULAR LESION, AND USE IN CARRIER DIAGNOSIS. J.F. Hejtmancik, J.D. Holcomb, W.C. Bromley, T.H. Roderick, M.C. Wilson, and R.A. Lewis, Baylor College of Medicine, Houston, Texas, Genentech, Inc., South San Francisco, and Center for Human Genetics, Bar Harbor, Maine.

Blue cone monochromacy is an infrequent retinal disorder characterized by poor central vision and color discrimination, infantile nystagmus, and nearly normal retinal appearance. Clinical detection of carrier females is not possible. Restriction fragment length polymorphism (RFLP) analysis was used in 3 multigenerational kindreds to establish linkage to 2 DNA markers, DXS15 (LOD = 3.58, 0 = 0.05) and DXS52 (LOD = 2.39, 0 = 0.07), both of which map near Xq28. Southern blot analysis with the red and green pigment genes reveals loss of rearrangement of the cone pigment cluster in all affected males examined, but in no family are all pigment genes missing. A combination of RFLP mapping and direct analysis of gene alterations has allowed carrier detection to be carried out in 8 females at risk for being heterozygous for this condition. Recombinant DNA analysis represents a generally applicable means for diagnosing carrier females and in some cases providing confirmation of the clinical diagnosis of blue cone monochromacy in affected males.

I 315 X-LINKED RETINITIS PIGMENTOSA: RETINAL FUNCTION ABNORMALITIES IN HETEROZYGOTES AND HEMIZYGOTES, Samuel G. Jacobson, Katsuya Yagasaki, Mark T. Chiu, Peter P. Apathy and Neal H. Raush, Bascom Palmer Eye Institute, Miami,FL 33136.

Detailed phenotypic characterization of patients with autosomal dominant retinitis pigmentosa (RP) using tests of retinal function has led to the discovery of different subtypes. We have applied such techniques to heterozygotes and hemizygotes with X-linked RP to determine if there were phenotypic differences within this genetic type.

Rod and cone ERGs, two-color dark-adapted static perimetry throughout the visual field and full clinical examinations were performed.

In heterozygotes, retinal dysfunction, when present, usually consisted of both rod and cone abnormalities. In individual eyes, there was a direct relationship between rod and cone ERG amplitudes and a negative correlation between rod amplitude and cone flicker timing. Frequently, there were significant interocular differences in ERG findings. Many patients had "patches" of retina with moderate or severe rod and cone sensitivity losses by perimetric testing.

Most hemizygotes had either non-detectable rod ERGs with barely detectable cone signals or non-detectable rod and cone ERGs. Cone-mediated central islands were separated by absolute scotomas in the midperiphery from rod-mediated islands in the nasal, temporal and inferior peripheral field. Rarely, however, patients showed greater cone than rod ERG abnormalities and one patient retained rod-mediated sensitivity in the central field.

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I 316 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE CODING FOR BOVINE ACIDIC FGF. Cécile Halley, Janine Altério, Jean-Claude Jeanny, Edith Jacquemin, Yves Courtois, Maryvonne Laurent, INSERM U.118, 29, rue Wilhem, 75016 Paris, France.

Fibroblast Growth Factor (FGF) exists under two closely related forms one basic (bFGF) and one acidic (aFGF). These mitogen peptides stimulate proliferation and differentiation of a wide variety of mesoderm and neuroectoderm derived cells, including many ocular cell types : epithelial lens cells, corneal endothelial cells. Autoradiography studies showed that these two FGF bind specifically to basement membranes of the eye. The lens capsule and the inner limiting membrane from retina are specially labelled by iodinated aFGF and bFGF. In an attempt to study the role of FGF in vision we constructed a cDNA library from poly A^+ RNA purified from bovine retina. cDNAs were ligated in the λ GTII expression vector and screened with synthetic oligonucleotide probes. Out of 2 10^6 recombinants one clone has been isolated and identified as coding for acidic FGF. The cDNA insert is 3700 bp long and includes the nucleotides coding for the 146 amino acids of aFGF. The translated region is flanked at the 5' and 3' ends by long untranslated regions. This cDNA clone hybridizes to two major mRNAs of 3,8 and 2,5 Kb and 2 minor ones of 9 and 6 Kb. Preliminary experiments using "in situ" hybridization show that in the neural retina mRNAs coding for acidic FGF are localized in the ganglion cell layer, the inner nuclear layer and the outer nuclear layer.

I 317 MOLECULAR MECHANISMS OF THE RETINOBLASTOMA GENE INACTIVATION IN RETINOBLASTOMA CELL LINE Y-79, Eva Y-H.P. Lee, Robert Bookstein, Chi-Jen Lin+, Lih-Jiuan Young and Wen-Hwa Lee*, Experimental Pathology Program and Center for Molecular Genetics*, Department of Pathology, M-012 and Department of Medicine+, University of California, San Diego, La Jolla, California 92093

The human gene (RB) determines susceptibility to retinoblastoma has been indentified recently by molecular genetic techniques. The RB gene encodes a 4.7 Kb transcript in normal tissues. In retinoblastomas, RB mRNA is either not detected or is expressed with altered size. Several retinoblastoma cell lines, such as Y79, RB 355 and WERI 27, belong to the latter group in that they all express shortened RB mRNA of 4.0 Kb. To understand the molecular basis for generating these small transcripts, we first characterized the well known retinoblastoma cell line Y79 in detail. A cDNA library was constructed from this cell line, and a cDNA clone was isolated using the normal RB cDNA as probe. Based on restriction enzyme mapping and DNA sequence analysis, an internal 471 nucleotide deletion near the 5' end of Y79 RB cDNA was identified. Moreover, about 2.5 Kb of 3' end cDNA is completely different from the normal RB cDNA. To further substantiate the mechanism of mutation, genomic clones encompassing the mutational junctions from Y79 and normal cell were isolated. Two clones from Y79 demonstrated intragenic deletion removing exons 2-5 from one RB allele, exactly corresponding to the cDNA deletion. Sequences from either end of the deletion had no apparent homology and did not contain Alu repeats. The data suggest the deletion was most likely via nonhomologous recombination. The 3' end divergence of Y79 RB cDNA occurs at an exon-intron junction. Sequence comparison of the corresponding normal and Y79 genomic clones revealed a three nucleotide deletion 15 basepairs away from the splicing donor site. The retaintion of intron sequences and further abnormal processing in the Y79 RB cDNA may result from this three nucleotide deletion and/or the major deletion at the 5' end. The deletion near the 5' end changes the reading frame and generates a premature termination codon. Consistent with this finding, no RB protein can be detected by immunoprecipitation with specific antibody. This study provides unequivocal molecular evidence that one RB allele in Y79 is multiply mutated and that a minimum of three mutational events has occurred.

I 318 DIAGNOSTIC POTENTIAL OF MELANOMA-ASSOCIATED ANTIGENS (MAA) IN THE VITREOUS OF PATIENTS WITH UVEAL MELANOMA, Peter E. Liggett, M.D., Malcolm Mitchell, M.D., Stephen J. Ryan, M.D., and June Kan-Mitchell, Ph.D., USC School of Medicine, Los Angeles, CA 90033.

Since only indirect methodologies are currently available to diagnose uveal melanoma, the shedding of selected MAAs in the vitreous of patients with uveal melanoma was explored as a novel diagnostic approach. The MAAs were identified by human monoclonal antibodies (MAbs) generated by fusing regional lymph node lymphocytes from patients with melanoma to a non-secreting mouse myeloma cell line. These MAAs appear to have a unique distribution; they are found in all melanoma cells studied, including 9/9 uveal melanoma specimens and 2/2 uveal melanoma cell lines. On the other hand, they are not expressed by other cells of neural crest origin, including benign skin nevus cells and melanocytes. Because these antigens are shed readily into the spent culture medium of the uveal melanoma cell lines established in our laboratory, we developed an enzyme immunoassay (EIA) for their detection in the vitreous of patients with uveal melanoma. Twenty vitreous samples were tested; 9 from patients with uveal melanoma, 4 from patients with proliferative vitreoretinopathy, and 7 from young trauma victims with no known ocular disease. All of the vitreous specimens from patients with uveal melanoma tested positive for the presence of these antigens but not in any of the other samples tested. These results demonstrate the presence of MAAs in the vitreous of patients with uveal melanoma. The ability to detect the MAAs in the vitreous of patients may form the basis for a useful diagnostic test in patients where a clinical diagnosis cannot be made. Further studies are needed to identify the limits of this test.

I 319 Susan J Lindsay*, D Jane Bower[1], Paul G Sealey[1], Helen Ackford* and Shomi S Bhattacharya*, *Molecular Genetics Unit, University of Newcastle, 19 Claremont Place, Newcastle-upon-Tyne, England, NE2 4AA; [1]MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, EH4 2XU, Scotland.

A study has been initiated to generate and map markers closely-linked to a number of human genetically-determined eye diseases. The following resources are being used: (a) cell lines from afflicted individuals with deletions in the region of interest; (b) hybrid panels containing defined regions of different human chromosomes; (c) existing probes and new probes isolated from cosmid and cDNA libraries; (d) pulsed field gel electrophoresis, to construct long-range maps of the target regions. Using the techniques of "reverse genetics", we plan to move from known chromosomal locations to the disease genes themselves.

I 320 ORGANIZATION AND EXPRESSION OF THE β-CRYSTALLIN GENES IN THE RAT.
H. Aarts, N.H. Lubsen and J.G.G. Schoenmakers, University of Nijmegen, Nijmegen, The Netherlands.
The βB2- and βB3-crystallin genes are closely linked in the rat genome. The βB1-crystallin gene, which was probably also once part of this cluster of basic β-crystallin genes, is located at least 30 kb away from the βB2- and βB3 genes. There is no correlation between the chromosomal organization and the developmental pattern of expression of these genes: the βB1 and βB3 transcripts reach their maximal concentration around birth, while the concentration of the βB2 transcript continues to increase till about 4 months of age. The transcripts from this gene account for about 90% of the β-crystallin RNA in the post-natal lens. A comparison of the β- as well as the αA- and αB-crystallin RNA levels during rat lens development with the published protein data suggests that control at the level of translation is an important regulatory mechanism in the expression of at least some of the crystallin genes.

I 321 LOCALIZATION OF THE GENE FOR XLRP TO Xp21 BY LINKAGE ANALYSIS,
Maria A Musarella, Rhoda Argonza, Arthur Burghess, Lynn Anson-Cartwright, Lap- Chi Tsui and Ron Worton, Hospital for Sick Children, Research Insitute, Toronto,Ontario,Canada.
X-linked recessive type of retinitis pigmentosa (XLRP) is considered the most severe form of RP with blindness occurring by the third or fourth decade of life. Linkage data for the location of XLRP on Xp and the phenotypic differences in female cariers suggest that there may be heterogeneity within the XLRP locus or a multiplicity of loci. Close linkage has been reported for XLRP with DNA probe L1.28 (Xp 11.3) as well as to the OTC locus at Xp21 for XLRP wiht metallic sheen. Localization of Xp21 is also supported by a pateint,"BB", with RP, Duchenne muscular dystrophy (DMD), chronic granulatous disease (CDG) and a deletion of Xp21.2. To locate the gene responsible for this disease, we have examined four families with multiple affected individuals by linkage analysis using RFLP probes spanning Xp21 to Xcen. Close linkage to XLRP was detected with DXS84 (754) (LOD max=3.71,θ = 0.05), OTC (LOD max=2.86, θ = 0.06) and DXS206 (XJ probes)(LOD max=2.13, θ = 0.11). The order of these loci is suggested by one individual who revealed a cross-over between XLRP and DNA markers DXS84 and OTC. This same individual and two others showed recombination between XLRP and DXS7 (L1.28) as well as XLRP and DXS14 (58-1), indicating that XLRP and DXS7 are distantly linked. Family data are consistent with the gene order: DXS28-DXS164-[DXS206-XLRP]-DXS84-OTC-DXS7-DXS14. Since DXS206, DXS84 and OTC are all located within Xp21, it is probable that at least one gene for XLRP also maps within this region.

I 322 LINKAGE ANALYSIS OF NORRIE DISEASE USING THE HUMAN ORNITHINE AMINOTRANSFERASE (OAT) cDNA PROBE.
Julielani T. Ngo, M. Anne Spence, Victoria H. Cortessis, Robert S. Sparkes, George Inana, and J. Bronwyn Bateman. Jules Stein Eye Institute and Departments of Ophthalmology, Psychiatry, Biomathematics, Medicine and Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024; National Eye Institue, National Institutes of Health, Bethesda, MD 20892. We have shown that the classic DNA marker for Norrie disease, the L1.28 probe, can recombine with the disease locus. In this study, we used a human ornithine aminotransferase (OAT) cDNA, which has been mapped to the same region on the X chromosome as the L1.28 probe to investigate the family with Norrie disease which exhibited the recombinational event. When genomic DNA from a family affected with Norrie syndrome is digested with Pvu II restriction endonuclease, we found the absence of 4.8 kb band in size which is most likely a result of a restriction fragment length polymorphism (RFLP) in the affected males. Further investigation indicates that this RFLP is probasbly generated by a point mutation and cosegregates with the disease locus with a positive lod score. These results suggest that one of the OAT-like sequences on the X chromosome may be in close proximity to the Norrie disease locus.

I 323 INDUCTION OF EXPERIMENTAL AUTOIMMUNE UVEORETINITIS (EAU) BY A SYNTHETIC PEPTIDE DERIVED FROM THE RETINAL PROTEIN IRBP. T.M. Redmond, H. Sanui, L.H. Hu, B. Wiggert, T. Kuwabara, G.J. Chader and I. Gery. National Eye Institute, NIH, Bethesda, MD 20892

Interphotoreceptor retinoid-binding protein (IRBP) is a ~140 KDa retina- and pineal-specific glycoprotein. Immunization with IRBP induces inflammation in the eye (EAU) and pineal (EAP) in various experimental animals. In an earlier study, we identified three CNBr cleavage fragments of bovine IRBP that induced EAU. These fragments have been localized to portions of IRBP of known amino acid sequence by N-terminal peptide sequencing and DNA sequence analysis. In order to identify the putative immunopathogenic epitopes within these fragments, ten synthetic peptides based on the two fully sequenced fragments were made and tested in rats for their capacity to induce EAU and EAP and to provoke immune responses. A 23 residue peptide, designated R4, was found to reproducibly induce EAU/EAP in immunized Lewis rats. This disease was less severe and less acute than that induced by intact IRBP. R9, a 27 residue peptide differing from R4 by the addition of 4 N-terminal residues caused a weaker disease than R4 and interacted very strongly with R4 in in vitro cellular response assays. Other tested peptides did not induce disease but some were found to provoke considerable cellular immune responses as measured by the lymphocyte proliferation assay. Additional peptides based on these fragments are being synthesized in order to further circumscribe the epitope represented in R4 and R9 and to localize other epitopes.

I 324 RETINAL PROTEIN GLYCOSYLATION IN POODLES WITH PROGRESSIVE ROD-CONE DEGENERATION, Maureen Rider and Dolores J. Takemoto, Kansas State University, Manhattan, KS 66506.

It has been previously reported that the rate of outer segment renewal in poodles with progressive rod-cone degeneration (PRCD) is reduced when compared to normal dogs. Western Blots, lectin-binding assays and two-dimensional peptide mapping were used to study differences in retinal proteins and glycoproteins in 6-week-old PRCD and normal poodles. Western analyses using polyclonal antisera revealed no differences in banding pattern of S-antigen, rhodopsin, transducin α or β, PDE γ or PDE $\alpha\beta$. There was slightly less reaction of rhodopsin and PDE γ and slightly more tropomyosin reaction in affected poodles. Lectin-binding assays with ^{125}I-peanut agglutin (Arachis hypogaea) revealed two bands at 25 Kilo dalton and 10 Kilo dalton in both normal and affected dog retinal homogenates. The lectin binding of the affected PRCD retinal proteins was reduced by 60% and peptide maps of these proteins revealed some differences. These studies suggest that while no change in protein patterns of typical retinal proteins occurs in PRCD poodles, there is a large decrease in the amount of protein glycosylation as detected by peanut agglutinin binding. This may cause a delay in membrane assembly and result in the observed reduced renewal rate.

I 325 DETECTION OF THE HSV GENOME IN HUMAN CORNEAL BUTTONS

BL Rong, KM Bean, EC Dunkel and DP Langston. From the Eye Research Institute and the Dept. of Ophthalmology, Harvard Medical School, Boston.

Corneal buttons of 46 patients were obtained at keratoplasty. Among these corneas, 14 were from patients with recurrent HSV keratitis and 32 were from patients with various corneal diseases (keratoconus, interstitial keratitis, aphakic bullous keratopathy, pseudophakic bullous keratopathy, graft rejection and Fuch's dystrophy). DNA was extracted from all corneal buttons seperately and DNA slot blot hybridization was performed with a [^{32}P]-labelled, cloned, full-length HSV DNA probe to detect the HSV-1 genome.

The HSV genome was detected in 7 out of 14 (50%) corneas from patients with recurrent HSV keratitis. At time of corneal transplant, all patients were in a clinically latent HSV infection stage (ie. no infectious particles were detected in the eye). These results indicate that the HSV genome can be retained in human corneas during quiescent, latent HSV infection and complement other electron microscopic and cultural assays on HSV detection in the cornea. The cornea may be an important site of extraneuronal latency in recurrent HSV keratitis.

I 326 LISCH NODULES AND LINKAGE ANALYSIS OF CHROMOSOME 17 PROBES IN THE LARGEST REPORTED KINDRED WITH NEUROFIBROMATOSIS I,

George O.D. Rosenwasser†††, Margaret Pericak-Vance†, Larry H. Yamaoka†, Arthur Aylsworth**, Jeffrey M. Vance†, Peter C. Gaskell†, Wu-Yen Hung†, Deborah V. Dawson††, Allen D. Rose†,*

**Bascom Palmer Eye Institute, University of Miami, School of Medicine, PO Box 016880, Miami, FL 33101, Duke University †††Department of Ophthalmology, Department of Medicine, †Division of Neurology, and †† Division of Biometry, Durham, NC, **University of North Carolina, Dept. of Pediatrics, Chapel Hill, NC.*

A prospective study of the largest known kindred with Neurofibromatosis I (NF-I) was undertaken to assess the diagnostic importance of Lisch nodules in the molecular genetic study of affected individuals. Complete ophthalmologic, neurologic and medical examinations were performed on 63 individuals of the 149 identified as possible carriers. In addition, DNA probes pE51, p10-41 and 17H8 were tested for linkage to the disease. Using the presence of Lisch nodules alone as a test for NF-I revealed an overall specificity of 97.1% with a sensitivity of 85.7%. Linkage was established for the disease to the pE51 locus and lod scores on RFLP linkage studies for p10-41 and 17H8 will be available at the time of presentation. Our Lisch nodule findings confirm previous studies on pooled patient populations while indicating that Lisch nodules are of marked importance in the early identification of individuals with NF-I. Linkage analysis demonstrates statistically significant linkage of the DNA probes tested to NF-I in the family studied. Correlation of the DNA results to the presence of Lisch nodules with updated lod scores will be presented .

I 327 CICLETANINE CURATIVE EFFECT ON EXPERIMENTAL MALIGNANT HYPERTENSION RETINAL DAMAGE, Marie-Madeleine Ruchoux, Marie-Therese Droy-Lefaix, Françoise Bakri, Joël Guillemain, Yves Lhuintre, Hôpital Bretonneau, Clinique neurologique, 37000 Tours, FRANCE

The effects of a new antihypertensive drug, the cicletanine (synthetic furopyridin), on retina changes were examined in stroke-prone spontaneaous hypertensive rats (SHR-SP)

Method : 39 SHR-SP/A3N Iffa-Credo (France) rats at the age of 11 weeks with hypertension lesions were randomly divided into 3 groups of 13 animals each : a control group and two groups treated with cicletanine 100 and 150 mg/kg/po/d. All the rats had free access to tap water containing 1 % NaCl. Blood pressure, body weight, survival were noted. After 6 weeks of treatment rats were sacrificed. Brain, heart, kidney samples were collected for morphological study. Posterior pole of the eyes were also removed for transmission electronic microscopic study.

Results : SHR-SP control group exhibited a rise in arterial blood pressure. Electron microscopic studies revealed changes of the retina vessels with a thickening of the endothelial layer surrounded by markedly large basal lamina. Multivesicular bodies were observed in the cytoplasm of the endothelial cells and pericytes of the retina vessels. In the nerve fiber layer, osmiophile deposition considered with so-called cystoid body was observed. On cicletanine treated SHR-SP rats, only minor ultra-structural vessels lesions were seen.

In conclusion, cicletanine inhibits the development of hypertensive retinopathy probably by its effect on the permeability of the retinal blood vessel wall.

I 328 *Abstract withdrawn*

I 329 EXPERIMENTAL UVEITIS INDUCED IN GUINEA PIGS WITH TWO SYNTHETIC OLIGOPEPTIDES, Vijay K. Singh, Kunihiko Yamaki, Larry A. Donoso* and Toshimichi Shinohara, National Eye Institute, NIH, Bethesda, MD 20814, *Wllis Eye Hospital, Philadelphia, PA 19107.

Experimental autoimmune uveitis (EAU) was observed in Hartley guinea pigs following the immunization with two small synthetic peptides, peptide M and peptide M15L, which correspond to the amino acid sequence of a well-characterized region of bovine S-antigen. Groups of guinea pigs were immunized with 100 µg of each peptide in complete Freunds' adjuvant and examined at regular intervals for the development of disease. Approximately two weeks later, an EAU was present which was characterized clinically by iris and pericorneal hyperemia. Histopathologically, a severe inflammatory response involving the uveal tract and retina was observed. In these eyes the photoreceptor cell layer of the retina was destroyed completely. A subretinal exudate containing mononuclear cells and polymorphonuclear leukocytes was also present. In addition, animals with EAU showed an associated pinealitis characterized by a lymphocytic infiltration of the subcapsular and central area of the pineal gland. Furthermore, draining lymph node cells of guinea pigs immunized with peptide M showed strong in vitro proliferative responses towards peptide M as measured by ^{3}H thymidine uptake. These results demonstrate the existence of at least one common pathogenic epitope in bovine S-antigen for the induction of EAU in Hartley guinea pigs and Lewis rats. Uveitopathogenicity and immunologic response of other oligopeptides will also be discussed.

I 330 INTERACTION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND CYTOMEGALOVIRUSES (CMV),Cindy Bohan**, Frank Shiao*, Rumei Yuan*, Henry J. Kaplan*, A. Srinivasan, Centers for Disease Control, Division of Viral Diseases, Atlanta, Georgia 30333; *Department of Opthamology, Emory University, Atlanta, Georgia 30333; ** Department of Medical Pathology, Emory University, Emory University, Atlanta, Georgia 30333. Acquired immune deficiency syndrome in HIV infected individuals is frequently complicated by central nervous system and ocular diseases. CMV retinitis is the major ocular complication of AIDS and is thought to be associated with human cytomegolovirus infection. Since HIV has been isolated from a variety of ocular sources and is shown to infect cells derived from ocular tissues, we have analyzed the interaction of human immunodeficiency virus and cytomegalovirus. Both human and murine cytomegalovirus were used for the studies. Cytomegaloviruses positively activate the expression directed by HIV long terminal repeat sequences in HeLa cells. The indicator plasmid pLTR-CAT contains HIV LTR sequences linked to chloramphenicol acetyltransferase. By constructing site-directed mutations in the LTR of HIV, it was possible to show the target sequences in the LTR essential for activation. The effect of immediate early gene products of CMV on HIV replication was also studied by transfecting cells with HIV proviral DNA. In addition, the influence of HIV transactivation gene products on CMV replication is also being analyzed to understand the role of HIV in CMV retinitis.

I 331 DETECTION OF HSV NUCLEIC ACID SEQUENCES DURING REACTIVATION IN RABBIT TRIGEMINAL GANGLIA. JF Stamler, CA Vaslet, KM Bean, EC Dunkel and DP Langston, Eye Research Institute, Harvard Medical School, Boston, MA 02114.

This study evaluates the activation of the specific HSV genome areas in rabbit trigeminal ganglion neurons after reactivation by corneal iontophoresis. Twenty-eight NZW rabbits were inoculated with 10^{5} PFU McKrae strain HSV-1. HSV infection was confirmed by slit-lamp examination and tear film culture. On day 60 post-inoculation, all animals except controls were induced to shed HSV by inontophoresis with 6-hydroxydopamine. Animals were sacrificed at 4, 6, 8, 12, 15 and 24 hours post-iontophoresis. Trigeminal ganglia sections were hybridized with [^{3}H]-labeled HSV DNA fragments representing Eco R1 map regions B, H or I (junction, unique long and unique short regions).

Non-reactivated ganglion sections hybridized with all fragments, B, H and I (1-3% of the neurons/section). 4 and 6 hours post-reactivation, hybridization was most intense with fragment H (3-4% of neurons/section). 8 and 12 hours post-reactivation, hybridization intensity was greatest with fragments B and I (1-2% and 2-3% of the neurons/section, respectively). 15 and 24 hours post-reactivation, sections hybridized with all fragments B, H and I, but fragment I showed the strongest reaction (1-3% of the neurons/section).

These results suggest a temporally regulated pattern of selective HSV genome expression during reactivation in rabbit trigeminal ganglia. They also confirm and extend indirect immunofluorescence studies using monospecific hyperimmune antisera to alpha, beta and gamma HSV polypeptides.

I 332 DIFFERENTIAL EFFECTS OF C-SRC AND V-SRC GENES, INTRODUCED INTO EARLY EMBRYONIC RETINA, ON HISTOGENESIS AND GLUTAMINE SYNTHETASE INDUCIBILITY. Lily Vardimon, Lyle E. Fox, Linda Degenstein and A. A. Moscona. The University of Chicago, Chicago, IL 60637

The cellular src gene (c-src) is expressed at high levels in neural tissues and is developmentally regulated in embryonic avian retina. Accumulation of c-src increases after the 6th day of development, peaks on day 11-13 and declines thereafter to a low level that persist in mature retina. The increase in c-src expression occurs during the period of cell differentiation and histological organization in the retina and it correlates inversely with cell multiplication.
The viral homologue of the c-src gene, v-src, is the oncogene of Rous sarcoma virus (RSV). The v-src gene product is responsible for transformation of cultured cells and for production of tumors in vivo.
In this study we used the RSV as a vector to introduce actively expressing v-src or c-src genes into early embryonic retina in organ culture. Embryonic retina tissue continues to develop in vitro comparably to in vivo. We found that expression of v-src at early embryonic stages prevented retina cells from undergoing normal histological organization; it also resulted in a high level of glutamine synthetase (GS) expression and prevented induction of GS by cortisol. In contrast, high levels of c-src expression at early embryonic stages did not affect histogenesis or GS incucibility.

I 333 DXYS1 AND DXS3 DO NOT FLANK THE GENE LOCUS FOR X-LINKED CHOROIDEREMIA (TCD). P.Wong, M.Tenniswood, & I.MacDonald, University of Ottawa, Ottawa, Ont, Canada.
The basic molecular defect underlying Choroideremia is not known. Subregional localization of TCD to Xq13-22 has been accomplished by linkage to two restriction fragment length polymorphisms, DXYS1 and DXS3; initial data indicated that TCD was closely linked (TCD:DXYS1 $\hat{\theta}$=0.00; TCD:DXS3 $\hat{\theta}$=0.03) to both loci (Nussbaum et al.,1985 Am.J.Hum.Genet 37:473-481; Lesko et al., 1985 Am.J.Hum.Genet 37:A65). We have examined three large choroideremia kindreds for segregation of these RFLPs in order to confirm these relationships. In the present study the LOD scores between TCD:DXYS1 peak at a LOD score ($\hat{Z}$) of 0.64 at a recombination fraction ($\hat{\theta}$) of 0.22 and TCD:DXS3 peak at $\hat{Z}$=3.98 at $\hat{\theta}$=0.14. In the case of TCD:DXYS1 full sets of LOD scores have been published (Nussbaum et al.,1985), addition of the present data to these shifts the TCD:DXYS1 $\hat{Z}$ to 5.23 at $\hat{\theta}$=0.05. In the three kindreds screened no DXYS1:DXS3 recombinants were identified and DXYS1:DXS3 LOD scores reached formal levels for the acceptance of linkage peaking at $\hat{Z}$= 2.71 at $\hat{\theta}$=0.00. In our linkage analysis two male offspring of a female carrier of choroideremia who was heterozygous for both DXYS1 and DXS3 with the phase of the disease and RFLP loci all known were observed. In both of these male individuals, TCD recombined with both DXYS1 and DXS3 without any evidence of recombination between DXYS1 and DXS3. It is therefore unlikely that DXYS1 and DXS3 flank the TCD gene locus.

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