Workshop: “Retinal Degeneration and Repair”
Wednesday, November 20, 2002

Twentieth Anniversary of the Ernest C. and Yvette C. Villere Chair for the Study of Retinal Degeneration

Louisiana State University Health Sciences Center
Neuroscience Center of Excellence and
Department of Ophthalmology

Organized and Chaired by
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We are indebted to Mr. St. Denis Villere and to Mrs. Margie Villere for their generous support of this celebration.
Welcome to the Workshop “Retinal Degeneration and Repair”

The scientists of the LSUHSC Department of Ophthalmology and the LSUHSC Neuroscience Center of Excellence are happy to be able to bring together leaders in the field of retinal research and look forward to the ideas, results, and scientific interaction that the participants of this Workshop on Retinal Degeneration and Repair are certain to bring forth.

Retinal Degenerations have become the largest cause of major visual loss in developed countries largely because no effective therapy exists for most types of retinal degeneration and also because their frequency and severity increase with age. Much research over many years by the participants of this workshop has led to understanding of pathophysiologic mechanisms and exciting possibilities for treatment.

We are grateful for the support of the National Eye Institute through competitive research grants and of the Eye, Ear, Nose, and Throat Foundation through the Yvette C. and Ernest C. Villere Chair for the Study of Retinal Degeneration.

The Ernest C. and Yvette C. Villere Chair for the Study of Retinal Degeneration was established in 1981 at the Eye, Ear, Nose, and Throat Hospital and occupied by Dr. Nicolas G. Bazan, who has been actively involved in retinal research for over thirty years and is recognized as an expert in the field of retinal degeneration and lipid neurochemistry. A native Argentine, he moved to New Orleans in 1981 and in a very short time established state-of-the-art laboratories and assembled an outstanding team of scientists at the LSU Eye Center. Dr. Bazan has distinguished himself by his work on retinitis pigmentosa and on several fundamental issues of the biochemistry of the retina. His work also extends towards understanding neuronal survival and its implications in neurodegeneration. The cross-fertilization of obtaining an insight on retinal degeneration by studying the brain and vice-versa is already happening in Dr. Bazan’s laboratory. Dr. Bazan organized and founded the LSU Neuroscience Center of Excellence in the same building where the Department of Ophthalmology is located. This has opened immense possibilities of collaboration between the Department and Center.

Grants from other institutions, particularly the National Eye Institute and National Institutes of Health, have strengthened the research funding of the Chair.

Donald R. Bergsma, M.D.
Professor of Ophthalmology
Director, Lions Eye Service
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Ernest Caliste Villere

Ernest Caliste Villere was born in New Orleans in 1904. After attending Tulane University, he went to work for Burroughs Adding Machine Company as a salesman. In 1927, he joined St. Denis J. Villere and Company, an investment counseling firm founded by his father in 1911. He became a partner a year later and remained there until his death at age 82 in 1986. Mr. Villere married Yvette Chequelin, and they raised two sons and a daughter, St. Denis, George, and Mathilde, who gave them thirteen grandchildren and four great-grandchildren.

Mr. Villere, who served as Vice-President as well as a member of the Board of Directors of the Historic New Orleans Collection, was an avid New Orleans historian. This is partly due to the fact that the Villere family played a major role in the establishment and growth of New Orleans. For example, his great-great-great-grandfather, Etienne Roy de Villere, accompanied Pierre Le Moyne, Sieur d’Iberville, on his first voyage to the Mississippi River in 1699, and his great-great-grandfather, Jacques Philippe Villere, was Louisiana’s first native Governor. The Governor’s son was the one who, after seeing troops approaching from the family plantation in Chalmette, warned General Andrew Jackson in New Orleans that the British were approaching the City.

Mr. Ernest Villere was instrumental in obtaining for the Historic New Orleans Collection the papers of Pierre Clement de Laussat, the French administrator who helped transfer the Louisiana Territory from France to the United States in 1803. Mr. Villere was also a member of the Society of the Founders of New Orleans, the Society of the War of 1812, and Society of the Sons of the American Revolution.

Throughout his lifetime, Mr. Villere served on numerous educational and governmental boards and committees, and was very active in the Roman Catholic Church. He was the founder and first president of the Financial Analysts of New Orleans, a member of the New York Society of Financial Analysts, a founder and treasurer of Valencia, Inc., a social club for teenagers, a treasurer of the Sierra Club, a founder and director of the Public Affairs Research Council and the Bureau of Governmental Research, a director of the Metropolitan Area Committee, the Information Council of America, and St. Mary’s Dominican College, and a trustee of the Holy Name of Jesus Parish. His philanthropic activities were acknowledged repeatedly, and he received many commendations and awards for service rendered to these various groups, including the Pro Ecclesia et Pontifice medal, an award for service to the Church and the Papacy, and the Order of St. Louis IX, King of France, for his work in the Archdiocese. He was the Vice-Chairman of the fund-raising drive for Hôtel Dieu Hospital, a trustee of the Alton Ochsner Medical Foundation and the Libby-Dufour Foundation, and a board member and secretary of the Eye, Ear, Nose, and Throat Hospital. He reigned as Rex, King of Carnival, in 1968.

Mr. Villere died November 1, 1986 at the age of 82.

Yvette Chequelin Villere

Yvette Chequelin Villere, a volunteer for numerous charitable organizations, was a lifelong resident of New Orleans. She attended the University of Wisconsin and graduated from Newcomb College in 1928. She studied social work at Tulane University in 1929 and participated in a summer program at the University of Sorbonne in Paris in 1931. She taught French at the Newcomb Nursery School and the Metairie Park Country Day School from 1930-35. She was a medical volunteer at Touro Infirmary Clinic, a volunteer social worker for the Bureau of Transients and Homeless, a board member and founding member of Valencia, chairwoman of the Women’s Division Community Chest in 1942, and a volunteer chairwoman for the Tulane and Newcomb Foreign Students Hospitality organization. She headed the Junior League Thrift Shop in 1934, headed the summer play session for the Junior League Community Center, headed the Junior Series for the Junior League, started a Cub Scouts organization at Holy Name School, started a Girl Scouts (Brownie) organization at Sacred Heart Academy, headed the Mercy Hospital fund drive, and started the Friday night square dances at Holy Name School.

Mrs. Villere died April 23, 1991 at the age of 83.
Dedication

Mr. Ernest C. Villere’s dedication and untiring efforts toward the establishment and advancement of retinal research led to the endowment of the Ernest C. and Yvette C. Villere Program on retinal degenerations of the Eye, Ear, Nose and Throat Hospital. A decisive force for the establishment of this Chair was the influence and inspiration provided by Professor Herbert E. Kaufman. Since 1981, I have conducted research supported by this program. It is both an honor and a privilege for me to occupy the Ernest C. and Yvette C. Villere Professorship of Ophthalmology and I am very grateful that I can use it for the betterment of humankind.

Mr. Ernest Villere’s keen insight and sensitivity allowed him to recognize the fact that retinal degenerations are a leading cause of blindness and that the only way to conquer these blinding diseases is through research. For it is only through studies such as those funded by the Chair that discoveries about the ultimate mechanisms of blinding disease are made. The findings from this research may lead to the development of effective treatments and perhaps even preventive regimens and cures for these devastating diseases.

I am deeply grateful to the family of Mr. and Mrs. Villere for all that they have done over the past years in support of Retinal Degeneration Research. Through their enthusiastic pursuit of funding for this program, as well as the family’s own generous donations, a significant amount of progress in this field has been accomplished. Mr. Villere had an uncanny understanding of the myriad details involved in launching a highly specialized research program that is rarely seen in people who are not scientists. The human greatness that he personified has inspired me and our research team to work even harder to achieve the goals that were so important to him. Our commitment has been strengthened and I aspire to return as much knowledge to humanity as I can through our research programs.

The friendliness, sensitivity, and creativity of Mr. Villere have been admired by all those whose lives he touched. Because of the personal quality of foresight he was a true visionary. The result of his vision is our research and the Neuroscience Center itself, a place where great strides have been made to alleviate some of humankind’s most tragic diseases. Although the world has lost a great benefactor and humanitarian, the death of Mr. Villere does not stop us all from benefiting from the fruits of his zealous campaign against blinding diseases. Mr. Villere served humankind well through his generosity and altruism; he continues to do so through the research performed under the auspices of the Villere Chair.

It is to Ernest C. and Yvette C. Villere that this workshop on “Retinal Degeneration and Repair” is dedicated. At the end of this program, I have included a list of selected publications of the work supported by the Chair.

Nicolas Bazan, M.D., Ph.D.
*Boyd Professor*
*Ernest C. and Yvette C. Villere Professor of Ophthalmology,*
*Biochemistry and Molecular Biology, and Neurology*
*Director, Neuroscience Center of Excellence*

*New Orleans, LA, 20 November, 2002*
HERBERT E. KAUFMAN, M.D. is Boyd Professor of Ophthalmology and Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center School of Medicine in New Orleans. He received his undergraduate degree from Princeton University and his medical degree from Harvard University. He interned at the Massachusetts General Hospital, after which he spent two years as a Fellow at the National Institutes of Health, followed by a residency in ophthalmology at the Massachusetts Eye and Ear Infirmary. He was the Chairman of the Department of Ophthalmology at the University of Florida in Gainesville from 1962 through 1977. In 1978, he moved to New Orleans, where he was Chairman of the Department of Ophthalmology and Director of the LSU Eye Center until April, 2001.

Dr. Kaufman developed the first effective antivirals, and pioneered the combination of steroid and antiviral therapy. He was involved in the development of M-K and K-Sol media, which made modern eye banks possible, the introduction of therapeutic soft contact lenses, and the development and clinical use of specular microscopy. He and his co-workers discovered that intraocular lenses can produce corneal endothelial damage, and that this damage can be prevented by viscous agents. He also helped to identify the beta adrenergic blocking agent, timolol, and was involved in the first clinical use of this drug to treat glaucoma. He has also been a leader in the development of refractive surgery in the United States.

He is a member of more than 30 professional associations and societies, including the American Academy of Ophthalmology, the American College of Surgeons, and the Royal Society of Medicine, England, and was the editor of Investigative Ophthalmology and Visual Science and served on the Editorial Boards of a number of journals, including the American Journal of Ophthalmology. He has been president of the Association for Research in Vision and Ophthalmology, the Contact Lens Association of Ophthalmologists, and the International Society of Refractive Keratoplasty and has served two terms on the Advisory Council of the National Eye Institute.

Among his honors are the Proctor Award (Association for Research in Vision and Ophthalmology, 1978), Pocklington Lecture (Royal College of Surgeons, London, 1979), XXXVI Edward Jackson Memorial Lecture (American Academy of Ophthalmology, 1979), the Twentieth Annual Edwin B. Dunphy Lecture (Harvard Medical School, Mass. Eye and Ear Infirmary Alumni Assoc., 1983), the First Annual Wohl Lecture in Ophthalmology (Rambam Medical Center, Haifa, Israel, 1983), the Annual Glover-Lisman Lecture (Manhattan Eye, Ear, and Throat Hospital, New York City, 1983), the R. Townley Paton, M.D. Award (Eye Bank Association of America, 1983), the G. Victor Simpson Lecture (Washington Hospital Center, Washington, D.C., 1984), the Peter Kronfeld Memorial Lecture (University of Illinois Eye and Ear Infirmary, Chicago, Illinois, 1984), the Kiewiet de Jonge Award (European Intraocular Implant Lens Council, Cannes, France, 1985), the Irvine Lecture (Doheny Eye Center, 1986), the Castroviejo Lecture (Castroviejo Society, 1987), the Montgomery Medal (Irish Ophthalmological Society, 1987), the first Gold Medal of the Saudi Ophthalmological Society (1989), the Earl Padfield Lecture (University of Kansas Medical Center, 1987), Innovators Award (American Society of Cataract and Refractive Surgery, 1990), the Ruedemann Memorial Lecture (Kresge Eye Institute, Detroit, 1990), the First Annual Claes Dohlman Lecture (Harvard Medical School, Boston, 1991), and the Jules Stein Living Tribute Award (RP International, Woodland Hills, California, 1992), the Award of Honor of the Association for Antiviral Therapy (1994), the Alcon Research Recognition Award (1997), a Lifetime Achievement Award from the International Society for Refractive Surgery (2000), and the Mildred Weisenfeld Award for Excellence in Ophthalmology from the Association for Research in Vision and Ophthalmology (2001).

He has more than 750 publications in his bibliography, including work on herpesvirus and ocular disease, antiviral drugs, corneal surgery, and refractive surgery.
Among Dr. Kaufman’s research accomplishments are:

1962: First antiviral drug effective for the treatment of virus infection

1964: Epithelial healing problems are due to hemidesmosomal defects

1964: Most effective antiviral developed to date

1965: Preservation of corneas suitable for penetrating keratoplasty

1969: Therapy of fungal infection with safe drug (Pimaricin)

1969: Recognition of glaucoma after penetrating keratoplasty

1970: Soft contact lenses introduced in U.S.A.

1970: First use of therapeutic soft lenses
Accurate measurement of intraocular pressure in eyes with irregular corneas or after surgery

1973: Simple, effective vitrector developed for anterior segment surgeon

1974: Introduction of McCarey-Kaufman (M-K) medium for corneal preservation

1976: First report of visualization of corneal endothelium in vivo

1976: First report of endothelial damage associated with insertion of intraocular lenses and use of viscoelastic substances as prevention

1977: First study of timolol for glaucoma

1980: Development of new refractive surgery technique: epikeratophakia

1984: Development of chondroitin sulfate-containing corneal preservation medium that permits storage of donor tissue up to 14 days

1987: Development of excimer laser refractive surgery

1988: Collagen shields for drug delivery to cornea
8:30 a.m.    Opening Remarks

8:45 a.m.- 9:15 a.m.    Marco Zarbin, M.D., Ph.D.
Professor of Neurosciences
Professor & Chair, Institute of Ophthalmology and Visual Science
Joint appointment as Associate Professor of Neurosciences
UMDNJ-New Jersey Medical School
Newark, NJ
“RPE Replacement for Age-Related Macular Degeneration:
Experimental Studies.”

9:15 a.m.- 9:45 a.m.    Nicolas G. Bazan, M.D., Ph.D.
Boyd Professor
Ernest C. and Yvette C. Villere Professor of Ophthalmology,
Biochemistry and Molecular Biology, and Neurology
Director, Neuroscience Center of Excellence
Louisiana State University Health Sciences Center
New Orleans, LA
“Photoreceptor Neuroprotection: fatty acids, lipid messengers,
and signaling to genes.”

9:45 a.m.- 10:15 a.m.    Robert Anderson, M.D., Ph.D.
Professor and Chair, Department of Cell Biology; Dean A. McGee Professor of
Ophthalmology
Adjunct Professor of Geriatrics and Biochemistry & Molecular Biology
Dean A McGee Eye Institute
University of Oklahoma Health Sciences Center
Oklahoma City, OK
“Activation of AKT in retinal Organ Cultures in Response to Insulin Mediated
through Phosphoinositide 3 Kinase”

10:15 a.m.- 10:30 a.m.    Break

10:30 a.m.- 11:00 a.m.    Connie Cepko, Ph.D.
Investigator, Howard Hughes Medical Institute
Head, Ph.D. Program in Biological and Biomedical Sciences
Professor, Department of Genetics
Harvard Medical School/HHMI
Boston, MA
“Genomics Approaches to Retinal Development.”
11:00 a.m.-11:30 p.m.  **Steven Fisher, Ph.D.**  
Professor of Neurobiology, Department of Cellular, Molecular & Developmental Biology  
Neuroscience Research Institution  
University of CA Santa Barbara  
Santa Barbara, CA  
“The Unexpected Capacity of Mammalian Retina for Neuronal and Glial Cell Remodeling.”

11:30 p.m.- 1:00 p.m.  Lunch

1:00 p.m.- 2:00 p.m.  **Chancellor’s Award Lecture in Neuroscience and Ophthalmology**  
**Paul Sieving, M.D., Ph.D.**  
Director  
National Eye Institute  
Bethesda, MD  
“Night-blindness and the State of Rod Photoreceptors in Retinitis Pigmentosa Rhodopsin Mutations”

2:00 p.m.-2:30 p.m.  **Robert Marc, Ph.D.**  
Professor of Ophthalmology and Physiology  
Department of Ophthalmology  
University of Utah School of Medicine  
Salt Lake City, UT  
“Neural Remodeling in Retinal Degeneration”

2:30 p.m.-3:00 p.m.  **Dean Bok, Ph.D.**  
Professor, Department of Neurobiology  
Dolly Green Professor, Department of Ophthalmology  
Member, Jules Stein Eye Institute  
University of California  
Los Angeles, CA  
“Gene-based therapeutic strategies for the treatment of inherited retinal disease in animal models”

3:00 p.m.- 3:30 p.m.  **John H. Wilson, Ph.D.**  
Professor, Department of Biochemistry and Molecular Biology  
Professor, Department of Molecular and Human Genetics  
Baylor College of Medicine  
Houston, TX  
“Knock-In Mouse Models for Therapy of Retinal Diseases.”

3:30 p.m.- 3:45 p.m.  Break
3:45 p.m.-4:15 p.m. **William Hauswirth, Ph.D.**  
Rybczki-Bullard Professor of Ophthalmology and Molecular Genetics  
Department of Ophthalmology  
University of Florida College of Medicine  
Gainsville, FL  
“AAV-vectored therapies for retinal neovascular diseases.”

4:15 p.m.- 4:45 p.m. **Joe G. Hollyfield, Ph.D.**  
Visiting Professor, Department of Ophthalmology, University of Puerto Rico  
Director, Department of Ophthalmic Research, Division of Ophthalmology, Eye Institute  
Joint Appointment, Department of Cell Biology, Research Institute  
Cleveland Clinic Foundation  
Professor, Department of Cell Biology, Neurobiology and Anatomy, Ohio State University  
Professor, Graduate Faculty, Kent State University  
Ophthalmic Research (i31)  
Cleveland Clinic Foundation  
Cleveland, OH  
“Proteomics of Isolated Drusen and Bruch’s Membrane: New Approaches to Understanding Age-Related Macular Degeneration”

4:45 p.m.- 5:15 p.m. **Matthew LaVail, Ph.D.**  
Professor of Anatomy and Ophthalmology  
Director, Retinitis Pigmentosa Research Center (Named Kearn Family Center for the Study of Retinal Degeneration in 1994), Department of Ophthalmology  
Beckman Vision Center  
UCSF School of Medicine  
San Francisco, CA  
“Models, Mechanisms and Therapy for Retinal Degenerations with Mertk Mutations”
Rod photoreceptors are light-sensitive cells within the retina with the remarkable property of remaining in a quiescent state in darkness until activated by light. The capture of a single photon of light by rhodopsin within rod outer segments activates the visual transduction cascade and results in vision. Rod photoreceptors play a central role in a set of human diseases termed retinitis pigmentosa (RP) which cause the death of the photoreceptor cells and lead ultimately to blindness. One of the first symptoms of RP is night-blindness, as the progressive degeneration and death of the rods impairs vision in dim lighting. RP diseases are inherited in families, and among the first genetic factors identified were mutations in the rhodopsin gene, the light sensitive molecule within the rod photoreceptor. In 1990, a pro23his rhodopsin mutation was found to co-segregate with vision loss in a large autosomal dominant RP family. Over the past decade, nearly 100 additional rhodopsin mutations have subsequently been identified as causing night-blindness and RP. Three of these rhodopsin mutations, including the gly90asp mutation, resulted in night-blindness, but without clinical signs of progressive vision loss. This suggested that a state of rod dysfunction could exist separate from rod degeneration and death. This talk will focus on the pro23his and gly90asp rhodopsin mutations in order to understand the consequences in the human disease state and the efforts to model these conditions in transgenic rodents.
“Photoreceptor Neuroprotection: Fatty Acids, Lipid Messengers, and Signaling to Genes”
Maria Soledad Cortina, William C. Gordon, Walter Lukiw, and Nicolas G. Bazan

Essential fatty acids play a critical role in photoreceptor cell function. Docosahexaenoic acid is a key building block of photoreceptors and arachidonic acid is the source of a cascade of potent lipid messengers that modulate cell function.

We are learning how fatty acids, lipid messengers, and signaling to genes regulate photoreceptor cell survival. One approach that is giving promising results is the development of novel low-molecular-weight compounds (LAU-0900 series) that elicit cone photoreceptor neuroprotection by down-regulating lipid signaling to certain pro-inflammatory genes.

We have begun to tease out signaling events that link mitochondria with DNA damage/repair. For this purpose we have studied bright-light-triggered apoptosis in rods. DNA fragmentation takes part in two waves, at approximately 24 hours apart, as shown by TUNEL, DNA SINE analysis, and DNA ladders. Photoreceptor loss was less than anticipated when 24- and 48- hr TUNEL was compared to cell loss after 10 days. Expression of DNA-repair enzyme polymerase β was up-regulated at 24 hr after light only in the region where cell loss occurred. Necrosis-like activity in cones was shown by EM. Cone damage peaked at approximately 6 hr after light with cone loss complete by 12 hr. Rod nuclei slowly degraded until chromatinolysis was complete at approximately 36 hr. RPE cells were healthy at 3 hr, but mitochondria began to swell by 4 hr. Mitochondria were the first to become affected, in the following sequence: cones, rods, RPE cells.

In conclusion, under bright light, a) rods are endowed with active DNA repair that is partially successful in rescuing damaged cells; b) this rod DNA repair may account for the two waves of fragmentation and lower-than-expected cell loss; c) necrosis-like cone cell death precedes that of rods; and d) mitochondria are affected early in cones, rods, and RPE. Therefore, signaling to sustain DNA repair may be critical for photoreceptor survival and these events may provide drug targets to promote photoreceptor survival and neuroprotection in retinal degenerations. (NEI grant number R01 EY05121)
“RPE Replacement for Age-related Macular Degeneration: Experimental Studies”

Chorioidal neovascularization (CNV) is the leading cause of severe vision loss in patients with age-related macular degeneration (AMD). CNV excision is technically possible, but few patients recover significant vision after surgery. Results in patients undergoing macular translocation indicate that RPE replacement might permit recovery of precision vision after CNV excision. Studies of RPE attachment to and wound healing on aged human submacular Bruch’s membrane organ culture provide a paradigm in which to develop RPE replacement strategies relevant to AMD patients. Results of RPE attachment studies indicate that differences in integrin expression may underlie, in part, the superior ability of cultured (vs. freshly harvested) human RPE cells to adhere to aged submacular Bruch’s membrane. Results of RPE wound healing on aged submacular Bruch’s membrane indicate that RPE resurfacing is impaired if wound healing must occur on the deep portions of the inner collagenous layers of Bruch’s membrane. We hypothesize that stimulation of proper RPE integrin and collagen receptor expression will improve RPE replacement success rates and visual outcomes after CNV excision.
Recently we have shown that phosphoinositide 3-kinase (PI3K) in the retina is regulated through in vivo light activation of the insulin receptor β-subunit. To try to determine the physiological relevance of this novel retinal finding, we cloned the 41-kDa cytoplasmic region of the retinal insulin receptor and we used the two-hybrid assay of protein-protein interaction in the yeast *Saccharomyces cerevisiae* to study the interaction between the regulatory p85 subunit of PI3K and the cytoplasmic region of the retinal insulin receptor. We found that p85 forms a specific complex with the cytoplasmic domain of the insulin receptor when both are expressed as hybrid proteins in yeast cells. We also determined that the interaction is strictly dependent upon receptor tyrosine kinase activity, since p85 shows no interaction with a kinase-inactive receptor hybrid containing a mutated ATP-binding site. These data suggest that the interaction between p85 and receptor is direct and provide evidence that tyrosine kinase activity is involved in these interactions. To validate our findings for yeast cells, we developed an organ culture system to study the activation of the retinal insulin receptor in response to insulin. The receptor activation is measured through GST-pull down assays with fusion proteins containing p85 full-length proteins, SH2 domains, or SH2 domain mutants. We found downstream activation of PI3K, Akt phosphorylation, and Akt activation measured by GSK-3α/β (Ser 21/9) phosphorylation. The insulin-mediated phosphorylation of GSK-3α/β (Ser 21/9) is inhibited by the PI3K inhibitor LY294002, suggesting that the PI3K pathway can be studied in this novel culture system. We suggest that the role of activation of the retinal insulin receptor may be to provide neuroprotection against light damage and other lethal stresses by activating proteins that protect against stress-induced apoptosis.
“Genomics Approaches to Retinal Development and Disease”

Our vision is dependent upon the proper development and function of photoreceptor cells in the retina, the thin sheet of tissue that lines the back of the eye that first interacts with light. Unfortunately, photoreceptor cells are prone to degeneration, particularly as we age, to give rise to such prevalent diseases as macular degeneration. We are studying how photoreceptors develop and degenerate using a variety of techniques. Most recently, we have used genomics approaches to find the genes that are expressed specifically in photoreceptor cells. We have identified approximately the full repertoire of genes expressed in photoreceptors. Among these 5-7,000 genes, we found that approximately 300 are expressed specifically, or nearly so, in photoreceptors. Of these 300, 264 are newly characterized, i.e. they were either undiscovered previously, or uncharacterized in terms of their expression pattern. Since many of the known photoreceptor disease genes are also photoreceptor-specific, we examined the map location in the human genome of these newly characterized 264 genes. Of these, 83 reside within the map locations of 33 human retinal disease loci for which there is not yet an identified disease gene. These 83 genes are excellent candidates for these disease genes. Our data also provide many candidates for genes that function in the generation and maturation of photoreceptor cells. We have also used these methods to characterize the genes that may play important roles in the generation and differentiation of other retinal cell types.
“The Unexpected Capacity of Mammalian Retina for Neuronal and Glial Cell Remodeling”
Steven K. Fisher and Geoffrey P. Lewis

Experimental retinal detachment and reattachment have revealed an unexpected degree of neuronal and glial cell plasticity in the mammalian retina. Using a variety of antibody probes in combination with imaging by laser scanning confocal microscopy we have demonstrated significant changes in all cellular layers of the retina in response to detachment and reattachment. Some of the most profound changes occur in Müller cells and these can have a direct impact on the recovery of vision because they can form a barrier to outer segmentation regeneration, when they form subretinal cellular membranes, or they can cause re-detachment of the retina when they form epiretinal membranes. These two events appear to have different initiating factors and occur through very different mechanisms. They each involve the segregation of different intermediate filament proteins into specific subcellular compartments as an early step. Vimentin, the surface molecule CD-44 and the growth factor, FGFII in concert with cone photoreceptors may play key roles in the subretinal growth of Müller cells. Retinal reattachment and GFAP appear to be key to their epiretinal growth. Remodeling of horizontal cells, bipolar cells, and ganglion cells all may play a role in the imperfect vision that often results after successful surgical repair of macular detachments.
Photoreceptor degenerations are progressive diseases with sequelae that transcend rod and cone loss. The neural retina is predominantly activated driven by cones of the sensory retina, even in rod-dominated mammalians. Though many inherited degenerations initially affect rods, secondary cone degeneration often ensues, resulting in sensory deafferentation of the neural retina. Using computational molecular phenotyping (CMP), we have discovered that most photoreceptor degenerations trigger a tertiary phase of remodeling that includes Müller cell hypertrophy and nuclear dislocation to both retinal margins; and neuronal migration, death, and formation of new neurite fascicles and neuropil. Fast photoreceptor degeneration models (e.g. TG9N mouse) display strong remodeling within 150 postnatal days (P), while slower systems such as the RCS rat and the GHL mouse do not express large-scale remodeling until P300. Even on this time scale, these phenomena mimic the progression of human retinal degenerations. Inversion of amacrine and bipolar cells to the ganglion cell layer and eversion of amacrine cells to the distal glial seal is accompanied disruption of laminar order in the inner nuclear and plexiform layers, representing large-scale corruption of retinal spatiotopy. Neuronal loss impacts all cell types, but genesis of new neuropil by survivor neurons is likely the most disruptive feature of remodeling. Extensive new wiring includes concatenated amacrine cell synapse chains as well as novel re-entrant bipolar cell loops that likely create unstable aperiodic or even oscillatory network behavior. Such rewiring is not uncommon in the central nervous system after deafferentation or sensory deprivation, but retinal rewiring is likely fictive or corruptive for visual processing. While remodeling is a barrier to cell-based and bionic rescue strategies, it represents a process that may yet be managed for retinal repair.
“Gene-based Therapeutic Strategies for the Treatment of Inherited Retinal Disease in Animal Models”

With a consortium of laboratories (M. LaVail, UC San Francisco; S. Nusinowitz, UCLA; A. Lewin and W. Hausrath, Univ. FL and D. Zack, Johns Hopkins) we are currently using three different gene-based strategies that employ recombinant adeno-associated virus (rAAV) vectors or inducible transgenes for treatment of inherited retinal disease. Our animal model is the *rds*<sup>-/-</sup> mouse with its spontaneous semidominant mutation or a dominant-negative P216L mutation on the *rds*<sup>+/-</sup> background.

Treatment of *rds*<sup>+/-P216L</sup> mice with rAAV that promotes the production of secratable human ciliary neurotrophic factor (CNTF) is effective in rescuing photoreceptors from cell death when a cytomegalovirus promoter or chick beta actin promoter drives expression. However, at high vector titers (>10<sup>12</sup> pfu and 10<sup>11</sup> pfu respectively) morphological rescue is not accompanied by functional rescue. Rod photoreceptor b-wave and a-wave sensitivity is decreased when compared to controls. Nuclear morphology of treated photoreceptors is also altered. Thus the effects of CNTF are pleiotrophic and dose-response issues are currently being addressed.

A second strategy involves the use of rAAV vectored constructs that provide ribozyme-based knockdown of mutant *rds*<sup>P216L</sup> mRNA and simultaneous replacement with wild-type mRNA to overcome semidominance. This project is currently underway.

Finally, windows of opportunity for gene therapy are being determined with the use of an inducible *rds*<sup>+</sup> replacement transgene on the *rds*<sup>-</sup> background. A minimum of cell rescue and induction of outer segment formation due to an activated *rds*<sup>+</sup> minigene can produce dramatic physiological improvement in the rod b-wave over a period of three months. Thus there is hope that partial rescue of photoreceptor structure and function can reap significant visual benefits for the organism.
“Knock-In Mouse Models for Therapy of Retinal Diseases”

Our goal is to create mouse models for studying the human rhodopsin gene, as a way of testing general strategies for gene-specific therapy of retinal diseases. We have constructed a visible form of rhodopsin by fusing the native human rhodopsin gene to the gene for green fluorescent protein (GFP), so that GFP is at the C terminus of the fusion protein. Gene targeting was used to replace the mouse rhodopsin gene with the human rhodopsin-GFP fusion gene in ES cells, and mice carrying the fusion gene in their germline were generated. Heterozygous knock-in mice express human rhodopsin-GFP at about the same level as mouse rhodopsin, have bright green retinas with GFP fluorescence localized to the rod outer segments, and retain normal retinal morphology. These mice will be useful for assessing treatments designed to decrease rhodopsin expression. In contrast to the heterozygous mice, the retinas in homozygous mice form normally and then degenerate. Abnormal light responses suggest that the human rhodopsin-GFP fusion gene is not functional; the biochemical basis for that deficiency is under investigation. Because fluorescence can be observed in intact animals, these homozygous mice offer the possibility for the live monitoring of treatments designed to slow retinal degeneration. To generate mouse models for gene correction, we have turned our strategy around and are knocking-in altered human rhodopsin-GFP constructs that do not express rhodopsin-GFP, and thus are not fluorescent. Treatments that correct a defect will turn on rhodopsin-GFP expression, which can be visualized as individual green rod cells against a colorless retina. To generate multiple knock-in mouse lines, we have devised a rapid method based on Cre/Lox site-specific recombination. These diverse knock-in mice should provide useful model systems for optimizing gene-specific treatments of retinal diseases, including for example, triplex-forming oligonucleotides, ribozymes, RNA/DNA chimeras, antisense oligonucleotides, and short interfering RNAs.
Pathologic ocular neovascularization (NV) is the primary cause of adult blindness in the developed world leading to Proliferative Diabetic Retinopathy (PDR) and Age-related Macular Degeneration (AMD). We asked whether AA V-mediated intraocular gene transfer of pigment epithelium-derived factor (PEDF) or kringle domains 1-3 of angiostatin (K1K3), both potent, well-tolerated anti-angiogenic agents, could inhibit the development of either retinal neovascularization or choroidal neovascularization (CNV) in standard animal models of each pathogenic process. By intraocular AA V-mediated gene transfer using a chicken beta-actin promoter/CMV enhancer to drive passenger gene expression, levels of PEDF or K1/K3 produced per adult mouse eye were well above estimated therapeutic thresholds for either agent. In a mouse model of CNV involving focal laser disruption of Bruch’s membrane leading to CNV, either intravitreal or subretinal injection of control AA V-GFP vector resulted in no significant change in the NV area compared to uninjected eyes. In contrast, either intravitreal or subretinal injection of AA V-PEDF resulted in significantly smaller areas of CNV over the same time course. Both treatments were effective for at least two months. In a retinopathy of prematurity mouse model of retinal NV that employs transient neonatal retinal ischemia to induce retinal NV, either AA V-PEDF or AA V-K1K3 administered intraocularly resulted in a significant qualitative reduction in retinal vascular proliferation as visualized by fluorescein angiography of the retinal vascular bed. By masked, pan-retinal sampling of retinal NV endothelial cells, either AA V-PEDF or AA V-K1K3 significantly reduced their number compared to either uninjected or AA V-GFP injected control eyes. Unlike adult mice in which measurable AA V vector passenger gene expression in the retina requires several weeks to reach its maximal level, such early ocular AA V vector administration resulted in therapeutic levels of gene expression within days. These data suggest that intraocular expression of secretable, anti-angiogenic proteins using AA V vectors may provide a viable, long-term treatment for the ocular neovascularization associated with Age-related Macular Degeneration and Proliferative Diabetic Retinopathy.
“Proteomics of Isolated Drusen and Bruch’s Membrane: New Approaches to Understanding Age-Related Macular Degeneration”

The causes of drusen formation and Bruch’s membrane thickening in age-related macular degeneration (AMD) are not known. Previous studies using immunocytochemical methods have identified some of the specific molecules present in drusen. However, to establish the full complement of molecules present, improved methods for drusen isolation and analysis are needed. We have developed microdissection procedures to isolate both drusen and Bruch’s membrane from donor eyes. Proteomic studies of these isolates from normal and AMD donors seek clues to understand the biochemical pathways involved in drusen formation, Bruch’s membrane thickening and AMD. Drusen protein was evaluated with SDS/PAGE, Western blotting and/or QTOF mass spectrometric analysis. Over 120 potential drusen proteins have been identified by LC MS/MS, 10% of which have been localized to drusen using immunocytochemistry. Newly identified drusen proteins include clusterin, calgranulin A, calgranulin B, psoriasin, annexin-I and annexin-IV. The most frequent peptides identified from trypsin digests of drusen are from TIMP-3, clusterin, vitronectin and serum albumin. Western blot analysis suggests that docosahexaenoate derived protein modifications (ie, carboxyethyl pyrrole adducts) are more abundant in AMD than in normal tissues. Abnormal protein cross-links also appear in drusen and Bruch’s membrane. Oxidative protein modifications may be causally involved in drusen formation and Bruch’s membrane thickening. The progression of AMD might be slowed if these processes could be modulated. Additional studies directed at this hypothesis are in progress.
An increasing number of retinal degenerations are becoming identified in which genetic mutations are expressed in the retinal pigment epithelium (RPE). A deletion in the receptor tyrosine kinase *Mertk* gene was identified in RCS rats through positional cloning methods, which suggested that *Mertk* was the retinal dystrophy gene that results in a failure of the RPE to phagocytize shed rod outer segments in these animals, thus leading to photoreceptor degeneration. A survey of human retinitis pigmentosa patients subsequently revealed underlying *Mertk* mutations, suggesting an importance of the Mer family of receptor molecules both in normal phagocytic mechanisms of the RPE and in some forms of inherited retinal degeneration. Further studies of the *Mer* mutations will be discussed, including reversal of the genetic defect and prevention of photoreceptor degeneration in RCS rats with gene-transfer methods, a new mouse model of retinal degeneration with functional knockout of the *Mer* gene, elucidation of phagocytic mechanisms of the RPE and consideration of possible new photoreceptor degeneration mechanisms.
SELECTED PUBLICATIONS SUPPORTED
BY THE
ERNEST C. AND YVETTE C. VILLERE CHAIR
FOR
RETINAL DEGENERATION
Selected publications supported by the Ernest C. and Yvette C. Villere Chair

1. Rodriguez de Turco EB, Parkins N, Jacobson SG, Bazan NG: Unexpected increase in plasma arachidonoyl phospholipid in retinitis pigmentos caused by Q64ter rhodopsin gene mutation. (in press)
20. Bazan NG, Rodriguez de Turco EB, Gordon WC: Docosahexaenoic acid supply to the retina and its


37. Bazan HEP, **Bazan NG**, Feeney-Burns L, Berman ER: Lipids in human lipofuscin-enriched subcellular


43. Scott BL, **Bazan NG**: Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc Nat Acad Sci*, 86:2903-2907, 1989.


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**Selected Publications on Neuronal Signaling**
