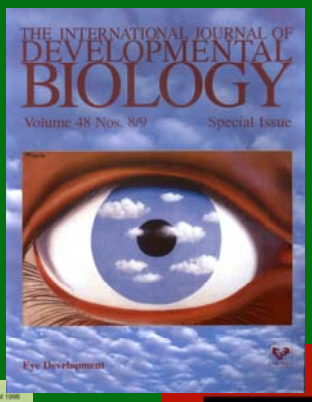
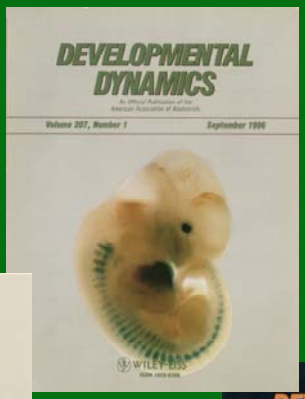
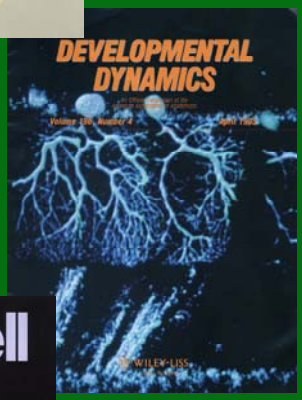
Laboratory of Molecular and Developmental Biology
25th Anniversary Symposium

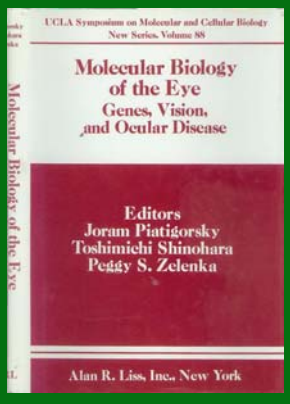
September 17 - 19, 2006

Lister Hill Auditorium, Building 38A NIH









Sponsored by the National Eye Institute, National Institutes of Health, Department of Health and Human Services

LMDB 25th Anniversary Symposium

NIH Campus, Lister Hill Auditorium, Building 38A

September 17-19, 2006

Agenda

Sunday, September 17

6:00 p.m. **Opening Meeting**

Hyatt Regency Hotel, One Bethesda Metro Center, Bethesda

(Light refreshments and beverages will be served)

Monday, September 18

NIH Campus, Lister Hill Auditorium, Building 38A

8:00 a.m. Coffee and light refreshments served

Introductory Remarks

8:30 a.m. **Carl Kupfer (former NEI Director)**

8:35 a.m. **Paul Sieving (present NEI Director)**

8:40 a.m. **Scientific Overview LMDB: Joram Piatigorsky (Chief, LMDB, NEI)**

Eye and Lens Growth

8:50 a.m. **Cheryl Craft : Moderator** (University of Southern California School of Medicine, Doheny Eye Institute, Los Angeles CA)

8:55 a.m. **David Beebe** (Washington University School of Medicine, St. Louis, MO)

9:20 a.m. **Peggy Zelenka** (LMDB, NEI)

9:45 a.m. **Melinda Duncan** (University of Delaware, Newark, DE)

10:10 a.m. **Ernst Tamm** (University of Regensburg, Regensburg, Germany)

10:35 a.m. Coffee Break (20 minutes)

Crystallin Gene Expression

10:55 a.m. **Ana Chepelinsky, Moderator** (FIC/NIH, NEI)

11:00 a.m. **Ales Cvekl** (Albert Einstein College of Medicine, Bronx, NY)

11:25 a.m. **Suraj Bhat** (UCLA School of Medicine, Jules Stein Institute, Los Angeles, CA)

11:50 a.m. **Eric Wawrousek** (LMDB, NEI)

12:15 p.m. **Lunch** (1 hour)

Eye Disease

1:15 p.m. **Venkat Reddy,** Moderator: (Kellogg Eye Center, University of Michigan, Ann Arbor MI)

1:20 p.m. **Toshimichi Shinohara** (University of Nebraska Medical Center, Omaha NE)

1:45 p.m. **Marc Kantorow** (Florida Atlantic University, Boca Raton FL)

2:10 p.m. **George Inana** (University of Miami School of Medicine, Bascom Palmer Institute, Miami FL)

2:35 p.m. **Teresa Borras** (University of North Carolina School of Medicine, Chapel Hill NC)

3:00 p.m. **Stanislav Tomarev** (LMDB, NEI)

3:25 p.m. **Group Photograph of all Participants**

3:30 - 5:00 p.m. **POSTERS** (Light refreshments and beverages will be served)

7:00 p.m. **Banquet,** Hyatt Regency Hotel

Informal Evening Speaker: **Joseph Horwitz, Other comments, as desired**

Tuesday, September 19

NIH Campus, Lister Hill Auditorium, Building 38A

8:00 a.m. **Coffee and light refreshments served**

Eye Disease and Industry

8:30 a.m. **Rashmi Gopal-Srivastava, Moderator** (OD, NIH)

8:35 a.m. **C. Richter King** (Gen/Vec, Inc., Gaithersburg, MD)

9:00 a.m. **C. Andrew Cuthbertson** (CSL Limited, Victoria, Australia)

Protein Structure and Function

9:25 a.m. **Vasilis Vasiliou, Moderator** (University of Colorado Health Sciences Center, Denver CO)

9:30 a.m. **Joseph Horwitz** (UCLA School of Medicine, Jules Stein Institute, Los Angeles CA)

9:55 a.m. **J. Fielding Hejtmancik** (OGVFB, NEI)

10:20 a.m. **John Nickerson** (Emory University School of Medicine, Atlanta GA)

10:45 a.m. **Coffee Break (20 minutes)**

Eye Evolution and the Diversity of Protein Functions

11:05 a.m. **James Jester, Moderator** (Eye Institute, University of California at Irvine, Irvine CA)

11:10 a.m. **Zbynek Kozmik** (Institute of Molecular Genetics, Prague, Czech Republic)

11:35 a.m. **Joram Piatigorsky** (LMDB, NEI)

Concluding Remarks

12:00 p.m. **Sheldon Miller** (Scientific Director, NEI)

LMDB 25th Anniversary Symposium

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Laboratory of Molecular and Developmental Biology

LMDB, NEI: A Brief Overview
Joram Piatigorsky

Happy 25th Birthday, Laboratory of Molecular and Developmental Biology!! However, lines are generally not so sharp in life; the seed begets the bud which precedes the flower. The LMDB is an outgrowth of a Section I headed (Section on Cellular Differentiation) in the Laboratory of Molecular Genetics (LMG; Chief, Philip Leder), National Institute of Child Health and Human Development, NIH for ten years. LMG investigators (Peggy Zelenka, Toshimichi Shinohara, Barbara Norman and I) were among the founding members of the LMDB. Suraj Bhat and David Beebe were in the Section on Cellular Differentiation, LMG, and left near the time when the LMDB was established; they have remained closely connected with the LMDB throughout its history and can certainly be considered "honorary" LMDB alumni. The high standards of LMG research established by Phil, with emphasis on individual creativity in an interactive and congenial environment, served as a valuable model for the LMDB.

Thanks are also due to Carl Kupfer (former Director, NEI) and Jin Kinoshita (former Scientific Director, NEI). The LMDB was created and supported for many years under their leadership. For me, the LMDB existed the moment Carl signed the letter on April 3, 1981, with Jin and me in his office, offering me the opportunity to form the new NEI laboratory. The letter stated "It is my purpose that this laboratory, dealing with molecular and developmental biology, should play a leadership role both nationally and internationally. Consequently, it will have the full support of the resources of the National Eye Institute in its growth and development." Actual transition to the NEI took one year and physical transfer to NEI laboratory space on the second floor of building 6 occurred in August, 1982. Technically, then, the LMDB is *almost* 25 years old at this symposium. The confidence of Carl Kupfer and Jin Kinoshita in LMDB potential was extremely important to us in sustaining the daily toils and occasional setbacks of our research efforts. I also take this rare opportunity to emphasize the academic freedom that Carl and Jin gave the LMDB. We were permitted to follow our instincts, and be driven by curiosity and opportunity to seek solutions to fundamental scientific problems. They accepted my only informal request - to be allowed to make "important mistakes" (not on purpose!). In return, Jin made one request to me: that *at least half* of the LMDB research concerns the eye! I assured him that *all* the research would concern the eye, and most of it would use the eye as a research tool. I strongly believe that long-range success and creativity of a research laboratory cannot be administered from the top down; it depends on great individual effort in an atmosphere of optimism and freedom. Thank you Carl and Jin for providing such an environment for the LMDB to flourish. Thanks are also due to J. Edward Rall (former Intramural Deputy Director, NIH) who encouraged me to establish the LMDB and provided advice and encouragement throughout the years, and to Paul Sieving (present NEI Director) and Sheldon Miller (present NEI Scientific Director), as well as Gerald Chader, and Robert Nussenblatt (preceding NEI Scientific Directors) for support with continued academic freedom.

In the 1970s the Section of Cellular Differentiation, LMG, NICHD laid the groundwork for future LMDB investigations by the following explorations: an embryonic chicken lens epithelial explant system (originally established by Dr. Alfred (Chris) Coulombre prior to the LMG) was used to study crystallin gene expression and lens fiber differentiation; lens opacities and crystallin synthesis were associated with ionic imbalances; crystallin mRNAs were purified, translated *in vitro* and quantified during early development (particularly chicken δ-crystallin mRNA); and the molecular genetics of crystallins was initiated by cloning and characterizing by electron microscopy δ-crystallin cDNA and genes.

At its first Board of Scientific Counselors' (BSC) review in 1984 the LMDB had four independent interacting groups (no Sections yet). The group leaders were Peggy Zelenka who was working on lens fiber differentiation and inositol lipid signaling, Toshimichi Shinohara who was working on opsin and S-antigen genes, Gabriel Vogeli who was working on collagen genes, and I who was working on crystallin genes, lens cell differentiation and cataract. Many others at this symposium who are now major contributors to NEI and vision research throughout the world joined the LMDB during these formative years. Already after a short four years and numerous publications, the LMDB was placing its alumni in significant scientific positions (for example, by the second BSC review in 1988, Toshi Shinohara joined the Laboratory of Immunology, NEI; George Inana and Teresa Borras joined the Laboratory of Mechanisms of Ocular Disease, NEI; John Nickerson joined the Laboratory of Retina, Cellular and Molecular Biology, NEI; and Gabriel Vogeli joined UpJohn in Michigan).

By its third BSC review in 1992 the LMDB had four Sections: Section on Cellular Differentiation (Peggy Zelenka), Section on Molecular Structure and Function (Graeme Wistow), Section on Transgenic Animals and Genome Manipulation (Eric Wawrousek), and Section on Molecular Genetics (mine). Ana Chepelinsky directed an independent group within my Section. Eric had been formerly a postdoctoral fellow in my Section, left to establish a transgenic mouse facility at Smith, Kline and French in Pennsylvania, and returned in 1989 to do the same for the NEI. His Section in the LMDB served then as now all NEI equally and upon need, and has been a resounding success story in ushering in transgenic and gene knockout technology for the NEI. In 1993, Ana was appointed Head of the Section on the Regulation of Gene expression. Stanislav (Slava) Tomarev came in 1989 from the N. K. Koltsov Institute of Developmental Biology, USSR Academy of Sciences in Moscow for a Sabbatical year in my Section, stayed on to become an invaluable member of the LMDB, and in 2003 was appointed Head of the Section of Molecular Mechanisms of Glaucoma in the laboratory. Today the LMDB comprises Sections headed by Peggy, Eric, Slava and me.

LMDB research has grown with the times and has always strived to remain on the forefront of new technology and ways of thinking, but the fundamental thrust from the beginning – to investigate the visual system at the molecular level while using the eye as a model for understanding the molecular, developmental and evolutionary genetics of eukaryotic cells – has remained its anchor.

Over the years there have been well over a hundred excellent scientists trained in or associated with the LMDB and probably close to 1000 LMDB-connected scientific publications comprising research articles, book chapters and reviews. The LMDB has also been mentor to a number of graduate and medical students officially receiving their doctorate degrees from U.S. or foreign universities (Johns Hopkins University, George Washington University, University of Michigan, University of Bonn, and presently University of Haifa) and undergraduate students who have gone on to obtain Ph.D. and M.D. degrees. I note with pride that although most recruits had little if any knowledge of the eye when they came to the LMDB, many have become leaders in different fields of vision research, including lens, cornea, retina and glaucoma. Moreover, although specialized in eye research, investigations performed by LMDB members and alumni invariably have broad significance and appear in leading journals of general interest. As indicated by the broad spectrum of eye research presented at this symposium, in addition to their strong basic science roots, these contributions often have relevance to immediate clinical problems such as cataract, corneal opacities or retinal degeneration and to more distant medical problems such as glaucoma and gene therapy.

There are many highlights of LMDB research accomplishments. I will mention just a few: Cloning and characterization of vertebrate and invertebrate crystallin genes, from man to jellyfish; establishing a direct relationship between the ex-

ons in the β/γ-crystallin genes and structural motifs in the encoded proteins; cloning of S-antigen and application of the concept of mimicry as a cause of uveitis; identification and detailed analyses of functional lens-specific and corneal-preferred promoters and enhancers in crystallin genes; initiation of genetic engineering in the eye in transgenic mice; knockout of crystallin genes in transgenic mice; discovery of regulation of crystallin gene expression by Pax6 and other transcription factors; extensive evidence indicating both divergent and convergent evolution of lens-preferred activity of crystallin promoters; discovery of some taxon-specific crystallins and enzyme-crystallins; development of the gene sharing concept; establishment of the concept of corneal crystallins and formulation of the refracton hypothesis based on gene sharing in the cornea; multiple studies on the role of proto-oncogenes, growth factors and signaling pathways in eye development; discovery of cdk5 as a lens and corneal protein regulating cell adhesion and migration; development of a transgenic mouse model for glaucoma; and discovery of optimedin and analyses of retinal gene expression associated with glaucoma. I regret the numerous omissions from this list, but there are just too many different contributions to be inclusive in restricted space due to the productivity of the LMDB. Many more areas have been and are being explored, such as global gene expression patterns in lens, cornea and retina, the role of microRNAs in corneal gene expression and development, and the multiple functions of corneal crystallins to name a few. LMDB scientists have also collaborated extensively at a national and international level and sent materials such as DNA clones, antibodies, cell lines and transgenic mice that they established to laboratories virtually all over the world, and these have been the source of innumerable and continuing scientific contributions.

The LMDB has populated the scientific community, especially but not only the vision science community, with well trained researchers here and abroad. Now at its 25th anniversary, LMDB alumni hold Professorships, Department Chairs, or Directorships (or the equivalents) in research institutions and industry worldwide. A smattering of examples are: Jules Stein Eye Institute at UCLA School of Medicine; Doheny Eye Institute at USC Keck School of Ophthalmology and Cellular Neurobiology; Yale University School of Medicine; Washington University School of Medicine; University of North Carolina School of Medicine; Albert Einstein School of Medicine; Emory University School of Medicine; Bascom Palmer Institute, University of Miami School of Medicine; University of Delaware; Florida Atlantic University; University of Kentucky; University of Nebraska Medical Center; Peking Eye Center (China); University of Regensburg School of Medicine (Germany); N.K. Koltsov Institute (Moscow); Institute of Molecular Genetics Czech Republic Academy of Sciences (Prague); INSERM, Paris (France); Tokyo Metropolitan Institute for Neurosciences (Japan); Institutode Biotechnologia (Mexico); Spic Science Foundation, Guindy, Madras (India); CSL Limited, Victoria (Australia); GenVec Inc, Gaithersburg; Minkon Biotechnology Inc, Gaithersburg; Dow, Indianapolis; Boehringer Ingelheim Pharma GmbH Co (Germany); and NIH. As for LMDB research accomplishments listed above, other research positions held by LMDB alumni are omitted from this list due to space constraints, however these are no less significant than those mentioned.

LMDB members and alumni have played key roles in promoting vision research throughout the world. These include: serving on national and international government, academic and industry panels providing scientific advice on matters of research priorities, funding and awards; being on the NEI Advisory Council (3 alumni); being on the ARVO Trustees and serving as Vice-President (1 member) and President (1 alumnus) of ARVO; delivering numerous invited seminars and keynote lectures at academic and industry institutions; organizing symposia and meetings (i.e. the first international meeting on the Molecular Biology of the Eye, UCLA Symposium on Molecular and Cellular Biology, Alan R. Liss, Inc. 1988); and compiling and editing accomplishments on eye research (i.e. Int. J. Dev. Biol. 48, Special Issue on Eye Research, 2004). With respect to awards, LMDB members and alumni have received accolades for scientific accomplishments. A selection of these are: the Jonas Friedenwald Award, the Proctor Medal, and the David Cogan Award from ARVO; the First Hans Bloemendal Lecture Award; the G. Burroughs Mider Lecture Award at NIH; 7 Alcon Research Institute Awards; the V. Everett Kinsey Lecture Award; 5 Senju/National Foundation for Eye Research Awards for Cataract Research; 2 Jin H. Kinoshita Lecture Awards of the National Foundation for Eye Research; the NIH Director's Award and 4 NEI Director's Award, among others.

We are all enriched by having many alumni remain in contact with the LMDB and continue to collaborate as a family. I have always considered "once a member of the LMDB, always a member of the LMDB." Perhaps this feeling of belonging has nurtured growth extending from the LMDB.

It is a nostalgic and meaningful experience for me to look back at these 25 years of the LMDB. With any endeavor there is always a nagging sense that there is so much more to do and so little time to do it in. Despite how much one desires perfection, one never comes close. But on balance, I am proud that the LMDB has come a long way and has impacted basic science in different areas, including of course the NEI and vision science. All members of the LMDB have cared greatly about their work, and their success is represented in part by the excellent lectures and posters that will be made at this symposium. The outstanding scientists performing significant research that, unfortunately, will not be presented at this symposium, again due to time constraints, are another mark of LMDB success: there is an impressive depth of activity and accomplishments with roots in the LMDB. If the next 25 years follow in the footsteps of the first, the future looks bright for the LMDB and its alumni.

I congratulate all of you and thank you for your efforts, loyalty and accomplishments. I am excited that so many of you have come to this symposium to celebrate our 25th anniversary and exchange scientific ideas as we have done so often in the past. In a phrase, the LMDB is all of us. I also thank the NEI for its generous and unflinching support of the LMDB in its many endeavors from the beginning, as promised initially by Carl Kupfer, for the continuing gift of academic freedom to pursue our work, and for sponsoring this symposium.

Laboratory of Molecular and Developmental Biology (LMDB) 2006

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LMDB 25th Anniversary Symposium

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Speaker Abstracts

Control of Postnatal Lens Growth by Hypoxia through HIF-1 and p27KIP1

David C. Beebe[1], Ying-Bo Shui[1,2] and Jeffrey Arbeit[3]

1. Ophthalmology and Visual Sciences, 2. Cell Biology and Physiology, 3. Surgery. Washington University, Saint Louis, MO 63110

The rate of growth of the lens slows with age. In rodents older than one month, this decline required the hypoxic environment normally present in the eye. Increasing intraocular oxygen did not alter the rapid cell proliferation in one-month-old lenses, but raising intraocular oxygen levels in four- to 11-month-old rats restored lens cell proliferation to the rate seen at one month. Increased epithelial cell proliferation led to the production of more lens fiber cells and faster lens growth. Cells respond to hypoxia by increasing the level of the transcription factor, HIF-1α. Conditional deletion of Hif-1a resulted in smaller lenses with abnormalities in cell differentiation, precluding their use for study of oxygen-dependent proliferation. Over expression of a modified form of HIF-1a that is not degraded in normoxia did not alter the basal rate of lens cell proliferation, but suppressed the oxygen-stimulated increase in proliferation. Increasing intraocular oxygen reduced the levels of the cyclin-dependent kinase inhibitor, p27KIP1 in older, but not in young lenses. This decrease was prevented in lenses that expressed stable HIF-1a. We conclude that the normal, age-related decline in lens growth is mediated by an age-related increase in the ability of HIF-1 to inhibit lens cell proliferation.

Transparency, Cancer and the Brain – on the function of Alpha B-crystallin

Suraj P. Bhat, Rajendra K Gangalum and Janice Caneria

Jules Stein Eye Institute, Geffen School of Medicine and Brain Research Institute, University of California, Los Angeles, CA 90095

Transparency of the lens, as it relates to crystallins, does not demand a specific protein, a specific structural domain or a fold within a protein. Crystallins are expressed in various tissues and are known to be associated with a variety of physiological and pathological processes. In addition to its presence in the ocular lens, alpha B-crystallin expression encompasses oncogenesis and neural degeneration. This brings up the question whether expression of a protein within the lens (as a crystallin) entails assumption of new/different functions specific to the lens? We have studied alpha B-crystallin gene expression and protein function within the ocular lens, as well as outside of the lens, in tissues and cells where transparency is not a phenotypic requirement. In non-transparent tissues/cells we find that alpha B-crystallin is associated with the perinuclear Golgi. Interestingly, much against the perception about lens cells as crystallin-crammed bags, we find that in the developing lens, almost all of alpha B-crystallin is associated with the Golgi. These comparable and similar findings in paradigms as diverse as the human glioblastoma cell line U373 and the ocular lens suggest a universal, non-crystallin (non-refractive) function for alpha B-crystallin within the lens as well as outside of the lens, in non-transparent tissues. A synthesis based on these ongoing investigations will be presented.

In search of Intraocular Pressure molecules

Terete Borrás[1], Jason L. Vittitow[2], Wei Xue3 and Nuria Comes[1]
1. Dept. Ophthalmology University of North Carolina, Chapel Hill, NC, 2. Inspire Pharmaceuticals, Durham, NC, 3. Northwestern University, Chicago, IL

Glaucoma is an optic neuropathy which causes irreversible blindness. Our approach to study glaucoma consists in selecting a well-established clinical condition associated with the disease and investigate the clinical phenomenon at the molecular level. Elevated intraocular pressure (IOP) is the major risk factor for the development of glaucoma. In humans, 90% of the aqueous humor exits the eye through the trabecular meshwork (TM), thus a dysfunctional TM results in increased resistance and elevated IOP. Because the physiology of a tissue is governed by the expression of its genes, we examine TM differential expression under conditions of elevated IOP. Pressure-perfused human anterior segment organ cultures, microarray analysis, TaqMan PCR, siRNA, viral gene transfer and calcification assays led us to the identification of several genes and mechanisms. Some were new to the TM, but intrinsic to unrelated tissues. In particular, Matrix GLA (MGP), inhibitor of osteogenic and cardiovascular calcification, and CDT6, a corneal antiangiogenic/ECM deposition gene were very abundant and upregulated by pressures insults. Following MGP's function, we find that the TM tissue undergoes a calcification process associated with age, glaucomatous conditions and lower MGP expression. Such process could be important in regulating resistance and bringing out potential new treatments for glaucoma.

An industry career based on proteins and the eye…

Andrew Cuthbertson, CSL Limited, Victoria, Australia

After medical and scientific training in Australia I was lucky enough to spend two marvellous years in the LMDB exploring gene sharing in the cornea. Feeling guilty about having so much fun, I moved to Genentech in 1992 to start their gene therapy lab working on factor VIII treatment for hemophilia A. While at Genentech I was drawn back to ophthalmology and applied recombinant monoclonal antibodies that neutralize VEGF to eye disease. We did early work exploring the role of VEGF in neovascular ('wet') age-related macular degeneration (AMD), and then developed high affinity, humanized neutralizing antibody fragments as the therapy which is now Lucentis™. In 1997 I moved home to head up R&D at CSL Limited, which is the biggest biopharmaceutical company in Australia. Highlights have been involvement with the development of Gardasil™, a recombinant vaccine developed by Merck for preventing cervical cancer, an avian flu vaccine and our recombinant antibody program. Current breakthrough treatments for AMD require intraocular injections. Most recently I have been able to return to my eye research roots by working with academic collaborators in Australia on topical delivery of recombinant antibody fragments for treating eye disease.

GENE REGULATION DURING LENS DEVELOPMENT: A GLOBAL NETWORK ANCHORED BY PAX6

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Early lens progenitor cells are formed during neural plate formation from a pool of partially-specified cells in the pre-placodal region (PPR) followed by formation of the lens placode. The earliest marker of the lens lineage is a "master" regulatory gene Pax6. Loss of Pax6 expression results in the absence of lens placode formation. During subsequent stages of lens formation, Pax6 activates expression of additional regulatory genes including Sox2, Six3, Mab21like1, Prox1 and c-Maf in lens. In turn, proteins encoded by these genes regulate expression of key lens structural proteins, crystallins. Recently, we dissected mechanism of temporal and spatial regulation of the mouse alpha A-crystallin locus suggesting that Pax6 and c-Maf participate in the recruitment of chromatin remodeling enzymes Brg1 and Snf2h, respectively. Conditional inactivation of Brg1 in lens disrupts lens fiber cell differentiation. In addition to lens, Pax6 also controls development of brain, pancreas and retina. We seek to determine both global and tissue-specific functions of Pax6 in these systems.

Deletion of the β1-integrin gene in the developing lens leads to epithelial mesenchymal transition (EMT) of lens epithelial cells
Melinda K. Duncan, Vladimir I. Simirskii, Yan Wang

β 1-integrins have been implicated in both normal lens development and posterior capsular opacification, however, their function in vivo has not been addressed. Mice harboring a floxed β 1-integrin gene were mated with MLR10-CRE mice to inactive the β 1-integrin gene in the entire lens. Mice homozygous for the floxed β 1-integrin gene harboring MLR10-CRE were microophthalmic, with little to no lens material detectable in adults. However, at 12.5 dpc, the lenses of these animals are morphologically normal with clearly distinguishable epithelial and fiber cells although no β 1-integrin protein is detected by immunofluorescence.. The lens capsule also forms normally with type IV collagen and laminin detectable in this structure. However, at 16.5 dpc, lens epithelial cell shape becomes abnormal, while at birth, the central lens epithelium is absent and the peripheral epithelial cells are undergoing apoptosis as assayed by TUNEL staining. α-smooth muscle actin expression is upregulated in the lens epithelium from 16.5 dpc onward, and the distribution of connexin 43 is disorganized consistent with epithelial-mesenchymal transition. These data suggest that β 1-integrin is not necessary for either the early morphogenesis of the lens nor the lens capsule. However, β 1-integrin is important to maintain the epithelial phenotype of lens epithelial cells later in development and its loss results in inappropriate epithelial-mesenchymal transitions.

A 5 base insertion in the γC-crystallin gene causes highly variable zonular nuclear cataracts through a direct toxic effect on lens cells
J. Fielding Hejtmancik, NEI, NIH

Highly variable autosomal dominant zonular pulverulent cataracts affecting 30 members of a seven-generation family are localized to an interval on chromosome 2q including the gamma-crystallin gene cluster. A 5-bp duplication within exon 2 of the γC-crystallin gene is found in each affected family member and is absent from unaffected family members and controls. This mutation disrupts the reading frame of the γC-crystallin coding sequence resulting in synthesis of an unstable γ-crystallin with 38 amino acids of the first "Greek key" motif followed by 52 random amino acids. In order to confirm this association and explore the mechanism by which this mutation might cause cataracts a mutant human γC-crystallin genomic clone driven by the chicken βB1-Crystallin promoter was expressed in transgenic mice. Lens fiber cells of transgenic mice show variable degrees of degeneration and vacuolization by 21 days of life. Western blot analysis of lens extracts confirms that the mutant protein is present in transgenic mice lenses. Real-time quantitative PCR reveals that levels of the HGC5bpd mRNA are similar to those of endogenous γC-Crystallin mRNA in normal lenses. Homology modeling suggests the mutant protein might exert a direct toxic effect, consistent with the histology of the transgenic lenses.

Alpha-Crystallin: Structure and Function
Joseph Horwitz, Linlin Ding, Qingling Huang and Xiaohua Gong

In spite of intensive work trying to elucidate the 3 dimensional structure of alpha-crystallin, we still do not have a high-resolution structure. This is because alpha-crystallin is polydispersed and cannot be crystallized. We can infer its structure from other small heat-shock proteins belonging to the alpha-crystallins family that were crystallized. To study its function in the lens, we are at present using a genetic approach. Xiaohua Gong and his collaborators have recently identified 2 mouse cataractous mutant lines. One such mutation is αA-R54C, and the second is αA-Y118D. The two mutations cause a recessive and a dominant cataract respectively. The first mutation (αA-R54C) which is in N-terminal region of alpha-crystallin seem to affect lens epithelial and fiber cell during development, the αA-Y118D mutation which is in the "α-crystallin domain" of the molecule affect the interaction of alpha-crystallin with the other major lens crystallins namely beta and gamma-crystallins. This approach and these kinds of animal models will help us understand the multiple roles of the alpha-crystallin in the lens and in the other tissues.

Strategies for molecular dissection of retinal degeneration

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Our research focus over the years has been to understand the mechanism of retinal degeneration with the hope of finding the best treatment or cure from the knowledge. A brief review of the various strategies taken to study retinal degeneration caused by single and multiple genes will be presented. For gyrate atrophy (GA), biochemical evidence of a defect in ornithine metabolism allowed us to clone the first gene, ornithine aminotransferase, for inherited retinal degeneration and identify mutations in GA patients. To identify additional single genes responsible for retinal degeneration, a subtractive cloning strategy was used to obtain retina-enriched genes that would be candidates for retinal degeneration. This strategy yielded 5 new retinal genes, 3 of which were demonstrated to cause retinopathies, including HRG4/UNC119, a photoreceptor synaptic protein, for which a dominant negative pathogenic mechanism involving mitochondrial ANT1 was elucidated through animal models. For age-related macular degeneration (AMD), a multi-gene complex disease, a gene expression profiling strategy using a custom macroarray enabled us to identify multiple candidate genes for further analysis and verification.

Protective and Repair Mechanisms in Age-Onset Cataract and Age-Related Macular Degeneration

Marc Kantorow, Department of Biomedical Sciences, Florida Atlantic University, Boca Raton, Florida.

Age-onset cataract and age-related macular degeneration are the leading causes of world blindness but to date little is known about the genetic and environmental factors leading to these diseases. Key to the development of these and other age-related diseases are free radicals generated through oxidative stress which are produced during aging. Two oxidative stress systems that we have discovered to defend and/or repair lens and retinal cells are the methionine sulfoxide reductases (Msrs), which are essential repair enzymes that govern entire lifespan in mammals, and the peroxiredoxins (Prxs) which are thioredoxin-dependent peroxidases that eliminate peroxides and free radicals in cells. Both of these enzymes are localized to the mitochondria and are essential for mitochondrial function. We have found that these enzymes protect lens and retinal cells against oxidative cell death. MsrA protects lens and retinal cells in vivo. We have also found that PrxIII is induced in the lens by as little as 2 µM H2O2 to levels 12 times above normal levels providing insight towards the development of potential therapeutics that could prevent or delay cataract, AMD and possibly other age-onset oxidative stress diseases. We thank the NEI, the American Health Assistance Foundation for Macular Degeneration Research and The Palm Healthcare Foundation for providing the funding that made this work possible..

Pre-clinical and Clinical Testing of PEDF for Ocular Disease

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Pigment Epithelium-Derived Factor (PEDF) is a potent anti-angiogenic with neuroprotective activity that is normally produced in the eye. PEDF protein delivered directly or via gene delivery can inhibit choroidal and retinal neovascularization in a variety of preclinical ocular models. Based on these findings, a phase I clinical trial was initiated in patients with severe wet AMD. Twenty-eight patients with advanced wet AMD were given a single intravitreous injection of a replication-deficient adenoviral vector with human PEDF (AdPEDF.11). Eight doses were investigated and no serious adverse events or dose-limiting toxicities were associated with AdPEDF.11. Early evidence suggests that PEDF has biological activity in inhibiting choroidal neovascularization in wet AMD patients. An expanded phase I trial in patients with less severe AMD had been conducted; accrual has recently completed.

Based on the positive findings from preclinical testing, and the early yet encouraging outcomes in the phase I clinical trial, we have continued research efforts to improve application of PEDF for eye disease. Since one of the major drawbacks to current therapies for wet AMD is the number of repeat intraocular injections (8-12 per year), we have identified three means to achieve prolonged PEDF protein activity in the eye.

EYE EVOLUTION: AN INSIGHT FROM CUBOZOAN JELLYFISH

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Cnidaria are the most basal phylum with a well-developed visual system. We have been exploring the cubozoan jellyfish (Tripedalia cystophora) containing complex, camera-type eye with retina, cellular lens and cornea. Our data suggest that an ancestral Pax gene was a primordial gene in eye evolution, regulating lens crystallins as well as opsin genes. We will present a tentative model explaining almost universal use of Pax genes for eye morphogenesis throughout the animal phyla. Finally, we have analyzed genes participating in the assembly of cubozoan eyes and found unexpected degree of genetic conservation as compared to higher animals. We propose that ancient cubozoan jellyfish are fascinating models for evo/devo insights into eyes and other sensory systems.

Potential functions of interphotoreceptor retinoid-binding protein (IRBP)

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I have been dogged by the simple question "But what does IRBP really do?" The current hypothesis is that IRBP serves as a high affinity reservoir for retinoids and fatty acids that dampens the rate of their movement from the RPE to photoreceptor cells. This hypothesis does not explain the phenotype of the knockout mouse, which has an unexpected morphological phenotype. Microarray and QTL analyses ought to identify genes associated with IRBP expression. An alternative strategy is the remarkable utility of cross-species studies among teleost fish. It is proposed that the ancestor of these fish underwent a whole genome duplication when the teleosts arose. During subsequent evolution the teleosts independently and differently compacted their genomes back to diploid from the original tetraploid state. The utility of studying the teleosts is that the compactions resulted in many different variants of the ancestral gene among the different teleosts. By sorting through these different gene and protein structures and looking at their different gene expression patterns, we then have a generally useful strategy to deduce the functions of a gene. I will illustrate the strategy with examples of IRBP genes from different species of fish. Because there are so many different teleost species, this provides a robust reservoir of possibilities, if you simply go fishing.

From Lens Crystallins to Gene Sharing.

Joram Piatigorsky, LMDB, NEI, Bethesda MD 20892-0704

Crystallins, as known to this audience, are abundant, diverse, taxon-specific, proteins with multiple functions in the lens and other tissues. The many roles of individual crystallins led to the concept of gene sharing, emphasizing (1) that a single gene can encode a protein with distinct molecular functions and (2) that gene regulation can dictate protein function. Corneas also have abundant, taxon-specific crystallins [i.e. aldehyde dehydrogenase 3A1 (ALDH3A1) in mammals and gelsolin/scinderin in zebrafish] that appear to serve multiple roles. For example, data suggest that mammalian ALDH3A1 participates in enzymatic detoxification, UV absorption, regulation of gene expression, cell growth and cell:cell interaction. Thus, lens and cornea are unified by accumulating crystallins and utilizing a gene sharing strategy. Multiple protein functions via gene sharing permeate the scientific literature, raising questions about defining and counting genes and implying, counter-intuitively, that specialization and diversification go hand-in-hand. Cross-talk between gene sharing and systems biology includes biological networks, molecular clocks, fluid boundaries for protein functions, cellular noise and variability, and medicine. Thus, gene sharing favors an open concept of genes and protein functions and appears to be a general principle of evolution.

Cataracts: Divergent Cataractogenic Stresses Share Common Death Pathways; The Unfolded Protein Response (UPR)
Toshimichi Shinohara, Dept. of Ophthalmology and Visual Sciences, UNMC, 985840 Nebraska Medical Center, Omaha, NE 68198-5840

It is becoming increasingly evident that common pathways are shared in the response to multiple, seemingly divergent cataractogenic stresses. In the highly oxidized lumen of the endoplasmic reticulum (ER), protein conformations are generated. If this process is disturbed by stress (ER stress), the UPR is induced. Our recent studies with ER or cataractogenic stressors in lens epithelial cell (LEC) cultures and in rats showed an induction of the UPR, production of reactive oxygen species (ROS), and induction of apoptosis in LECs, and the development of cataracts in lenses.

Although a transcriptional factor, ATF4 activates the expression of CHOP, a central death transcriptional factor of the UPR, ATF4 also activates the expression of lens epithelium-derived growth factor (LEDGF) in LECs. LEDGF is a survival factor and transcriptional activator of the stress protective genes. Thus, up-regulation of the ledgf gene suppresses the UPR dependent apoptosis. During the lens fiber differentiation, CHOP is also expressed and LEDGF is suppressed. Transcription of the ledgf gene can be regulated by cross talks with other bioactive ligands such as IGF-1, TNF-α, and TGF-β. Investigation of the UPR and cross talks with the other signaling pathways in cataractogenesis and lens fiber differentiation will be discussed.

Sox11 plays an important role during lens formation
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Background: Early eye development is under control of a variety of transcription factors. In the present study, we investigated the role of the high-mobility-group transcription factor Sox11 for lens formation.
Methods: The ocular phenotype of Sox11-/- mice was investigated from embryonic day (E) 9.5 until birth. BrdU-labeling was used to study cell proliferation. Transient transfection experiments were used to study the influence of Pax6 on Sox11 expression.
Results: Lenses of Sox11-/- -deficient mice did not separate from the surface ectoderm during early development and showed structural changes at birth. The defects in lens vesicle formation were associated with reduced mitotic activity in the lens epithelium. In some of the animals, the lens failed to develop completely. The expression of Sox11 was reduced in Pax6-/- mice, and was enhanced in vitro when cells were transfected with an expression vector for Pax6. In Sox11-/- mice, the expression of BMP7 was markedly reduced during anterior eye development. Conclusions: Sox11 is required during separation of the lens vesicle from the surface ectoderm. The expression of Sox11 is under control of Pax6. Changes in BMP7-signalling are involved in the effects of Sox11 on anterior eye development.

Olfactomedin domain-containing protein: from glaucoma to neural differentiation.
Stanislav Tomarev, SMMG, LMDB, NEI

A family of olfactomedin domain-containing proteins consists of at least 13 members in mammals. Using different model systems, we investigate properties of three olfactomedin domain-containing proteins, which are expressed in the eye: olfactomedin 1, optimedin, and myocilin (Myoc). To develop a genetic mouse model of glaucoma, we produced Myoc knockout mice, as well as transgenic animals, expressing normal and mutated Myoc in eye drainage structures. Only animals expressing mutated Myoc developed eye defects (moderate elevation of intraocular pressure, degeneration of retinal ganglion cells and optic nerve) resembling open-angle glaucoma in humans. To study possible functions of optimedin and olfactomedin we used PC12 cells as a model system. We demonstrated that optimedin and olfactomedin affects NGF-induced differentiation of PC12 cells in opposite directions: olfactomedin stimulates axon growth while optimedin inhibits axon growth and stimulates cell aggregation through the pathway involving N-cadherin activation. In zebrafish, inhibition of olfactomedin expression induced multiple developmental defects including reduced size and pigmentation of the eye and body, and cell detachment from the head and dorsal body. Our data support the idea that olfactomedin domain-containing proteins may play important roles in normal development and diseases.

Roles of α-crystallin in development and function of lens and other tissues

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The small heat shock protein family members αA- and αB-crystallin are present at enormously high levels in lens, while αB is present in most tissues at modest levels, and αA is present only in small amounts in select non-lenticular tissues. Many functions have been ascribed to these proteins, including: heat shock protein, molecular chaperone, cytoskeletal binding protein, autokinase, cell cycle regulator, antiapoptotic agent, clinical predictor of tumor treatment success, oncogene, etc. They have been shown to be involved in a variety of diseases including cataract, myopathies, cardiomyopathies, neurodegenerative diseases such as Alexander's disease and Alzheimer's disease, and scores of others, with the list growing continuously. Gene knockout of the αA and αB genes in mouse has provided useful tools for probing the essential roles of α-crystallin in vivo. Use of these models has demonstrated that: αA is essential for lens transparency, and solubility of high concentrations of αB in the lens; αB is not essential for lens development or transparency; αB is critical for maintaining structure and function in select skeletal muscles; αB is not essential for heart development or function under non-stressed conditions, but plays an important role during and following ischemia/reperfusion stress; absence of αB in cultured lens cells allows hyperproliferation (cell cycle role); loss of αA or αB render RPE cells more susceptible to apoptosis in response to oxidative stress; loss of both α-crystallins leads to opaque lenses with developmental abnormalities and degradation of secondary fiber cells; αB neither initiates nor exacerbates inflammation of nerves in multiple sclerosis. We have only begun to recognize the many functions of these versatile proteins

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Cdk5 Regulates Cell Migration in Lens and Corneal Epithelium

Peggy Zelenka

Proper control of migration is essential for normal development, morphology, and healing of epithelial tissues. Directed migration is a complex process, which requires coordinate regulation of lamellipodial extension with cytoskeletal contraction, formation and disruption of cell-matrix adhesions, and dynamic changes in cell-cell adhesion. The serine/threonine kinase, Cdk5, is a key regulator of these processes in the lens and corneal epithelium. Cdk5 must bind one of two related activating proteins, p35 or p39, to become enzymatically active. These activators also direct Cdk5 to its substrates through specific protein-protein interactions. We have identified a novel interaction between the activating protein, p39, and myosin essential light chain, which may position Cdk5 to phosphorylate regulatory proteins involved in myosin contraction. Cdk5 also promotes assembly of stress fibers and strengthens adhesion to fibronectin in lens and corneal epithelial cells. These effects require direct binding of Cdk5 to Src. Cdk5 has an equally important role in stabilizing cadherin-dependent junctions. Suppressing Cdk5 expression with siRNA reduces immunostaining of E-cadherin junctional complexes in corneal epithelial cells, while inhibiting Cdk5 activity reduces corneal epithelial cell adhesion to E-cadherin ectodomain and increases E-cadherin degradation. Thus, Cdk5 regulates multiple pathways allowing it to coordinate disparate events for efficient migration.

Poster Abstracts

POSTER 1

Serial Analysis of Gene Expression (SAGE) in the Rat Limbal and Central Corneal Epithelium

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Purpose. To identify genes preferentially expressed in the stem cell-rich limbal epithelium of the rat cornea. Methods. SAGE libraries were constructed from the limbal and central corneal epithelial cells, and in situ hybridization, RT-PCR and cDNA cloning were conducted. Results. The rat limbal and central corneal epithelial SAGE libraries consisted of 41,894 and 40,691 tags, respectively. After annotation this reduced to 759 transcripts specific for the limbal library and 844 transcripts specific for the central corneal library; 2,292 transcripts overlapped. In situ hybridization and/or RT-PCR results of 12 of the most abundant highly enriched transcripts in the limbal epithelium were in general agreement with the SAGE data, and also showed that these proteins are also expressed in the conjunctival epithelium. Interesting limbal-enriched transcripts encode WDNM1-like protein (similar to WDNM1/Expi, a putative secreted proteinase and inhibitor of metastasis), mesothelin (a cancer marker), marapsin (a trypsin-like serine protease), K4 and K15 (both cytokeratins). WDNM1-like protein was cloned and confirmed as a member of the 4 disulfide core family. Conclusion. The SAGE results extend the database of genes expressed in the rodent cornea and suggest an association between several genes preferentially expressed in the limbal epithelium with cellular proliferation and migration.

POSTER 2

Deciphering structure & function of visual arrestins in cone photoreceptors

Cheryl M. Craft

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Both visual arrestins, arrestin1 (ARR1/S-antigen/rod) and cone arrestin (CARR/ARR4/X-ARR), are co-expressed in cone photoreceptors and have light-dependent translocation to outer segments and bind to photoactivated, phosphorylated S- and M-opsins (J. Neurosci. 2003, 23:6152). The function of visual arrestins in cone phototransduction shutoff is still unknown. The X-linked CArr-/- founder mice were created and genotypes verified. CArr-/- was backcrossed onto the Arr1 KO background (provided by J. Chen, Nature 1997, 389:505) for double KOs (DKO). Specific epitope antibodies for ARR1 (monoclonal, Mab D9F2, Donoso) or CARR (polyclonal, Pab LUMIj) identified distinct expression patterns in light and dark in these KO models. Successful germline transmission of CArr was verified by PCR genotyping and CArr+/- cones had reduced levels of expression. Dual localization of ARR1/CARR confirmed expression in cones that was absent in DKO retinas. Photopic ERG analysis of CArr-/-, Arr1-/- and CArr-/-Arr1-/- mice for cone response was dependent on the environmental light exposure and the cone b-wave amplitudes were altered. Both visual arrestins may contribute to the shutoff of the light-activated, GRK1 phosphorylated S- and M-opsin pigments; however, because of altered b-wave responses, other functions are being examined in the cone synapse.

Grant Support: CMC is the Mary D. Allen Chair in Vision Research, DEI; Mary D. Allen Endowment; EY015851; and EY03040 (DEI Core)

POSTER 3

Differential expression and chromosomal arrangements of the α-crystallin genes in zebrafish.

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There are two αB-crystallin (αB1, αB2) and one αA-crystallin genes in zebrafish. Here we investigate their expression patterns during development by whole mount in situ hybridization and in the adult by semi-quantitative RT-PCR. αA-crystallin gene expression appears in the lens at 24, 48 and 72 hpf (hrs postfertilization), in the brain at 24 hpf, and in the otic capsule at 72 hpf. αB1-crystallin gene expression appears in the brain at 24, 48 and 72 hpf and in the heart at 48 and 72 hpf. αB1-crystallin expression was not detected in the lens until 6 dpf (days postfertilization). αB2-crystallin gene expression was detected in the lens, heart and brain at all stages. In adults, αA and αB1-crystallin expression appear lens-specific while αB2-crystallin expression is widespread. NCBI entrez gene tool analysis shows that the αB1-crystallin gene is located on chromosome 15 head to head with the Dixdc1 gene and the αB2-crystallin gene on chromosome 21 head to head with the predicted HspB2 gene. Similar chromosomal arrangements exist in mice and chicken. The gene expression patterns coupled with the chromosomal arrangements are consistent with a co-orthologous relationship of the zebrafish αB1- and αB2-crystallin genes with the single mouse αB-crystallin gene.

POSTER 4

ALDH3a1 is Regulated by Pax6, OCT1, and p300

Janine Davis, Barbara Norman, Dionne Davis, and Joram Piatigorsky, NEI, NIH, Bethesda, MD

Aldehyde dehydrogenase 3a1 (ALDH3) is an abundant corneal-enriched mRNA and protein whose expression is developmentally regulated. We have investigated mechanisms regulating the expression of ALDH3a1 by cotransfection of plasmids containing portions of the ALDH3a1 promoter fused to a reporter gene with selected transcription factors (IOVS.2004, 45:429).

The mouse -2537/+3486 ALDH3a1 promoter is upregulated ~10-fold by cotransfection with Pax6. Cotransfection with p300 did not alter ALDH3a1 promoter activity; however, with the addition of Pax6, promoter activity was increased 30-fold. OCT1, a classical POU domain protein, increased ALDH3a1 promoter activity 46- and 115-fold when transfected alone or with Pax6, respectively. The ALDH3a1 promoter is activated nearly 200-fold in the combined presence of Pax6, OC T1, and p300. In vivo, ALDH3 expression is downregulated in SEY mice.

Functional analysis of truncated ALDH3a1 promoter constructs implicated several Pax6 and OCT1 binding regions. Pax6 binds a variant paired domain site at -85/-46 and OCT1 binds two sites at -803/-779 and +3434/3458, in vitro. Although Pax6 and OCT1 colocalize to corneal epithelial cells, we were unable to detect a direct interaction between Pax6 and OCT1.

POSTER 5

Designer Antimicrobials: Peptidoglycan Hydrolase Module Shuffling.

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Bovine mastitis (mammary gland infections) exists on every dairy farm, affects 1/3 of the animals, and costs the US dairy industry about $2 Billion annually. There are multiple mastitis-causing pathogens but streptococci and staphylococci are the most predominant. Broad spectrum antibiotics are often less than 50% effective, with culling often necessary. Transgenic cattle resistant to S. aureus mastitis challenge have been created via mammary gland expression of a peptidoglycan (PG) hydrolase (lysostaphin) that is lytic for S. aureus cell walls (Nat. Biotechnol. 23:445-51). Phage endolysins are a prodigious source of novel PG hydrolases that often contain two discrete enzymatic domains that each target a unique bond in the PG. We have performed deletion analysis and site-directed mutagenesis to show that the streptococcal phage B30 endolysin Acm glycosidase (lysozyme-like) domain, although highly conserved and enzymatically active on cell wall preparations, does not contribute significantly to cell lysis. Fusions of PG hydrolase domains have been shown to maintain their domain-specific hydrolytic activities. Fusing either the highly active B30 CHAP endopeptidase domain or the full length B30 endolysin to full length lysostaphin creates an antimicrobial that is active against both S. aureus and several streptococci. This designer antimicrobial approach demonstrates the facility of constructing highly specific antibacterial proteins that target multiple unrelated pathogens simultaneously.

POSTER 6

Localization of Pdlim2 at cell-matrix adhesion sites requires an intact actin cytoskeleton and Rho activity

Chun Gao, Sandy Sun, Stanislav Tomarev, and Peggy Zelenka

LMDB/NEI/NIH, Bethesda, MD

Pdlim2 cDNA, encoding a protein containing an N-terminal PDZ motif and a C-terminal LIM domain, was previously identified in a rat eye iridocorneal angle cDNA library. It is highly expressed in the corneal epithelium and is associated with α-actinins, filamin A, myosin VI, and MYH9. In the present study, we further explore its potential involvement in cell adhesion, motility, migration, and actin cytoskeleton in Human Corneal Epithelial Limbal cells (HCLE) by Total Internal Reflection Fluorescence Microscopy (TIRF). The results showed that GFP-Pdlim2 was specifically localized to cell-matrix adhesion and actin attachment sites at the periphery of cells grown on fibronectin. Control cells transfected with GFP only did not acquire any specific TIRF signals. Cytochalasin D treatment completely obliterated Pdlim2-associated structures at the cell periphery, while treatment with C3, a Rho family inhibitor, changed the distribution of Pdlim2 from cell adhesion sites to lamellipodia. Immunofluorescence showed co-localization of Pdlim2 with actin cytoskeletal filaments and phospho-S19 myosin light chain regulatory chain (MLC II) at the cell periphery. We also identified active Rho, Pdlim2, phospho-S19 MLC II, and β1 integrin in protein complexes isolated by Rhotekin RBD affinity chromatography. Consistent with these findings, immunofluorescence of corneal whole mounts detected Pdlim2 protein only in the corneal epithelia in association with actin cytoskeletal structures. These findings demonstrate that localization of Pdlim2 at cell adhesion sites requires an intact actin cytoskeleton and Rho activity, and suggest that Pdlim2 plays a role in corneal epithelial cell adhesion and migration.

POSTER 7

The role of FGF Signaling in Lens Growth and Development.

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Fibroblast growth factors (FGFs) are implicated in lens development. In mice and humans, there are 22 FGF ligands and four FGF receptors (FRs). We used conditional and germline knockouts to study the roles of FGFs during mouse lens development. Lenses lacking FR1 or FR3 were normal. Lenses lacking FR2 were small, had increased apoptosis in epithelial and fiber cells and increased BrdU incorporation in fiber cells. Lenses lacking FR1and FR2 had a more severe phenotype; lenses were very small and in some cases showed no primary fiber cell differentiation. We also discovered that FR4 is found in lens epithelial and peripheral fiber cells. Lenses lacking FR4 were normal in embryos and adults. Lenses lacking FR2 and FR4 had the same phenotype as lenses lacking FR2. We conclude that no single FR is required for lens induction or the formation of epithelial and primary fiber cells. FR2 is required for withdrawal of fibers from the cell cycle and for survival of lens cells. FR1 and FR2 are necessary for primary fiber cell formation. There are no apparent genetic interactions between FR4 and FR2. We are examining other potential genetic interactions by performing triple and quadruple knockouts of the FGF receptors.

POSTER 8

THE ZEBRAFISH CORNEAL CRYSTALLIN IS ENCODED BY A NOVEL GENE FROM THE GELSOLIN SUPERFAMILY

S. Jia[1], V. Vasiliou[1], J. Kanungo[1], M. Spencer[1], Donita Garland[1], Marina Omelchenko[2], Eugene Koonin[2] and Joram Piatigorsky[1].

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A gelsolin-like protein (initially called C/L-gelsolin) comprises ~ 50% of the water-soluble protein in the cornea and exists in lesser amounts in the lens of adult zebrafish. Zebrafish also possess a second gelsolin-like protein (initially called U-gelsolin) with a broad tissue expression pattern. Genomic and phylogenetic analyses of the gelsolin superfamily revealed that zebrafish corneal crystallin, C/L-gelsolin, is encoded by a novel gene paralogous to the mammalian gelsolin and scinderin genes. Zebrafish U-gelsolin appears orthologous to mammalian gelsolin. In addition, zebrafish contain a separate ortholog to mammalian scinderin. C/L-gelsolin appears to lack a mammalian ortholog and is paralogous to the zebrafish gelsolin and scinderin genes. The U-gelsolin and C/L-gelsolin genes have duplicates in zebrafish. We therefore named the C/L-gelsolin gene gsnl1a and its duplicate gsnl1b, and the U-gelsolin gene gsna and its duplicate gsnb. There appears to be a single scinderin gene (called scin) in zebrafish. The expression profiles of the five genes (gsnl1a, gsnl1b, gsna, gsnb and scin) were studied using RT-PCR, and the major corneal proteins were separated with 2D-gel electrophoresis and identified with tryptic digestion and MALDI/TOF mass spectrometry. The data confirm that gsnl1a is the corneal crystallin in zebrafish.

POSTER 9

The role of Pax transcription factors in eye evolution

Iryna Kozmikova and Zbynek Kozmik

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Anatomically widely different designs of animal eyes have long been thought to arise independently multiple times during evolution. This view has been challenged about a decade ago by the landmark discoveries that a highly conserved transcription factor Pax6 plays a key role in eye morphogenesis both in flies and mammals. Since then, more evidence has emerged for redeployment of Pax6 and some other developmental control genes in the genetic programme underlying eye formation throughout the animal kingdom. We will present a tentative model, based on the complex nature of Pax transcription factor that explains an almost universal use of Pax genes for eye morphogenesis throughout the animal phyla.

POSTER 10

Cataract Phenotype in Aldh1a1-/-/Aldh3a1-/- Double Knockout Mice

Natalie Lassen[1], J. Bronwyn Bateman[1], Tia Estey[1], Jer R. Kuszak[2], Joram Piatigorsky[3], Gregg Duester[4], and Vasilis Vasiliou[1]

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Purpose: The aldehyde dehydrogenases ALDH3A1 and ALDH1A1 are expressed at high concentrations in the cornea and lens of the mammalian eye. Our long standing hypothesis is that these proteins protect the eye against environmentally-induced corneal injury and cataract formation. To test this hypothesis we have developed an Aldh1a1-/-/Aldh3a1-/- double knockout mouse line in order to examine possible structural and functional changes in the eyes of these mice.

Methods: Single and double knockout mice were evaluated for structural changes in the cornea and lens by slit lamp biomicroscopy and scanning electron microscopy. The expression of several antioxidant enzymes (i.e. catalase and Cu-Zn superoxide dismutase), aldehyde-detoxification enzymes (i.e. alcohol dehydrogenase and aldose reductase) and other corneal crystallins were studied in all genetic stocks by Western blot analysis. The chymotrypsin-like activity of the proteasome was evaluated using a fluorogenic substrate in all mice. Finally, 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA)-adducted proteins were detected by Western blotting.

Results: Aldh1a1-/-/Aldh3a1-/--deficient mice exhibited corneal abnormalities detected by scanning electron microscopy. In addition, these mice developed lens opacification in the anterior and posterior subcapsular regions as well as punctuate opacities in the cortex that were detected by slit lamp biomicroscopy. No changes in the expression pattern of these proteins were found in either cornea or lens of double knockout mice compared to wild type animals. Significant inhibition of the chymotrypsin-like activity of the proteasome as well as an increase in 4-HNE- and MDA-protein adducts were observed in the double knockout mice compared to the wild type mice.

Conclusions: These findings support a novel function for ALDH3A1 and ALDH1A1 in the protection of proteasome activity by metabolizing toxic aldehydes produced during lipid peroxidation. The double knockout mouse line we generated highlights the importance of ALDH1A1 and ALDH3A1 in corneal and lens physiology and provides a valuable model to study the combined roles of these enzymes in clinically-significant diseases of both cornea and lens.

Supported by NEI EY11490 and EY08282 Grants.

POSTER 11

Blind mole rat αB-crystallin promoter function in transgenic mice.

Yan Li, Barry Hough, and Joram Piatigorsky. LMDB, NEI, NIH, Bethesda, MD 20892-0704

The subterranean blind mole rat (Spalax galili) has subcutaneous, regressed eyes. We have shown the -668/+45 mole rat αB-crystallin promoter fragment has little lens and high muscle activity in transgenic mice compared to its mouse counterpart, despite high sequence homology. Here we show the -968/+45 promoter sequence comprising the full intergenic region between the mole rat aB-crystallin and Hsp myokinase binding protein gene also lacks lens activity in transgenic mice, consistent with the blind mole rat promoter undergoing tissue-specific modifications during its adaptation to underground life. Gel mobility shift assays using nuclear extract from both lens cells and myoblasts showed the -274/-262 mole rat sequence and its mouse counterpart bind distinct nuclear factor(s). We mutated the -273CA nucleotides in the mouse αB-crystallin promoter to G, as occurs in the mole rat promoter. The mutated mouse promoter has low lens but high muscle activity in transgenic mice. Moreover, the gel mobility shift pattern of the mutated mouse sequence resembled that of the mole rat sequence. Finally, supershift assays suggest the -274/-262 mole rat promoter sequence may interact with the Sp1 transcription factor. Our data suggest the -274/-260 blind mole rat promoter sequence contributes to

the selective loss of the lens activity.

POSTER 12

αA-CRYSTALLIN INHIBITS CASPASE-9-DEPENDENT PROCESSING OF PROCASPASE-3.

Viktor Morozov, Eric F. Wawrousek.

Laboratory of Molecular and Developmental Biology, NEI, NIH.

We recently showed that lenses of α-crystallin-null mice are opaque, and secondary fiber cells undergo an apoptosis-like disintegration process likely involving caspase-3 and -6. Inhibition of caspase-3 maturation by αB-crystallin was reported previously, however little is known about the effect of αA-crystallin. Our data with α-crystallin-null mice suggest that αA-crystallin plays a similar, or even more important, role than αB-crystallin in inhibition of caspase activity. Processing of procaspase-3, by caspase-9, in the presence of α-, αA-, or αB-crystallin in a cell-free system resulted in 10 fold or greater inhibition of caspase-3 activity compared to experiments in which crystallins were absent. Cleavage of procaspase-3 by caspase-9 in the presence of αA-crystallin resulted in formation of 26 kDa polypeptide. Pull-down assays demonstrated that, under our experimental conditions, caspase-9 interacts with αA-crystallin, but not with αB-crystallin. Inhibition of caspase-9-dependent procaspase-3 maturation by αA-crystallin, and interaction between caspase-9 and αA-crystallin suggest a role for αA-crystallin in regulating apoptosis. In secondary lens fiber cells, α-crystallin interferes with the apoptosis-like maturation program, halting it after organelle removal, but before cellular disintegration. Our data, along with data of others, suggest that α-crystallin interferes with the caspase cascade in the apoptotic pathway triggered by cytochrome c/ATP.

POSTER 13

Zebrafish Olfactomedin1 regulates neural network in developing eye and brain.

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1) LMDB, National Eye Institute/NIH
2) LMGD, National Institute of Child Health and Human Development /NIH, Bethesda, MD.

Olfactomedin1 (Olfm1/Noelin/Pancortin) is a secreted glycoprotein implicated in neurogenesis and generation of the neural crest. Expressions of Olfm1 mRNA and endogenous protein in zebrafish were analyzed by wholemount in situ hybridization, Q-PCR, immunofluorescence and western blot. In the course of development, zebrafish Olfm1a and Olfm1b, orthologs of the mouse Olfm1 gene, were expressed in neurons in the telencephalon, hindbrain, and dorsal and ventral spinal cord. Trigeminal ganglia and pronephric ducts also strongly expressed Olfm1 mRNA. Olfm1-MO injection caused damage to the brain and eyes, accompanied by cellular dissociation from the head and the dorsal part of the body, reduced pigmentation, and an expanded heart. Axon extensions in the optic nerve, lateral line nerve, and neuronal network inside of the neural tube were strongly inhibited by the Olfm1-MO injection. Expression of snail1, slug and microphthalmia-associated transcription factors (mitfs) were dramatically changed by Olfm1-MO. The expression of their common target gene, e-cadherin (e-cad), was reduced in embryos treated with Olfm1-MO, possibly contributing to the cellular dissociation and the damage in brain and eye. These data suggest that zebrafish Olfm1 may affect neuronal and eye development through the modulation of expression of genes including e-cad, slug, snail1, and mitfs.

POSTER 14

Expression of RP58, a novel zinc finger transcriptional repressor, in developing mouse brain

Chiaki Ohtaka-Maruyama and Haruo Okado

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RP58, a novel zinc finger protein containing a POZ domain, functions as a sequence specific transcriptional repressor. RP58 gene disrupted mice show severe abnormalities in brain cortical layer formation, suggesting that RP58 has a crucial role in cerebral development. To understand the role of this protein in brain development, we examined RP58 gene expression in mouse embryo and adult brain by in situ hybridization.

As a result, we found that RP58 transcripts are first detected at embryonic day 10 in the neuroepithelium of the spinal cord and telencephalic vesicle. In the day 12-13 embryos, RP58 transcripts are predominantly observed in the preplate region but not in outside the nervous system. At E15, RP58 transcripts were detected throughout the neocortex and hippocampus, but not in the thalamus and striatum. In the cortex, the transcripts were detected primarily in cortical neurons, but not in the marginal zones and ventricular zone. In adult mice, RP58 is expressed in neocortical and hippocampal neurons and granule cells in the cerebellar cortex.

POSTER 15

Expression Profiles of MicroRNAs During Mouse Cornea Development

Jennifer Pitrak, Donita Garland and Joram Piatigorsky, NEI, NIH

MicroRNAs (miRNAs) are a class of small endogenous noncoding RNAs that posttranscriptionally down regulate gene expression and play important roles in diverse processes such as embryonic development, differentiation, and cancer. In association with the RNA-induced silencing complex (RISC), miRNAs either cause target mRNA cleavage and degradation or inhibit mRNA translation. To examine the role of miRNAs in mouse corneal development, corneal miRNA expression profiles were evaluated by microarrays in post-natal day 6 (PN6) and 6-week-old (adult) mice. Forty-two miRNAs demonstrated at least a 2-fold difference in expression between the two developmental stages. Of these differentially expressed miRNAs, upregulated miR-184, miR-31, miR-205, and miR-204, and downregulated miR-199b were corneal-enriched when compared to a heart miRNA expression profile and known miRNAs. miR-184 is the most abundant miRNA in adult mouse cornea and miR-31 demonstrates the largest change in expression for those miRNAs upregulated in adult. Quantitative real-time PCR analysis confirmed the miRNA expression profile data. To find corneal miRNA targets, protein profiles of PN9 and adult corneas are being compared by 2-dimensional gel electrophoresis. Consideration of corneal miRNA expression in light of corneal SAGE, microarray, and protein profile data may yield insight into miRNA targets and the roles miRNAs play in corneal development.

POSTER 16

Cdk5 binds Directly to Src to regulate stress fiber formation in lens epithelial cells

Fengyu Qiao, Chun Y. Gao. Peggy S. Zelenka, NEI, NIH

Previous studies from this laboratory have shown that Cdk5, a kinase known primarily for its neuronal functions, is expressed in active form in the lens and promotes cell-matrix adhesion in cultured lens and corneal epithelial cells. Here, we explore the molecular mechanism underlying the effect of Cdk5 on adhesion by examining the relationship between Cdk5 and Src. Cdk5 co-immunoprecipitated with Src from rabbit lens epithelial cells (N/N1003), indicating that these proteins form a complex intracellularly. In vitro studies with GST-tagged Cdk5 or c-Src showed that these two kinases directly interact, and located a strong binding site in the N-terminus of Cdk5. Binding of Cdk5 to c-Src was independent of Cdk5 activity or phosphorylation status, as shown by IP experiments with specifically mutated Cdk5 constructs. To examine the physiological consequences of the Cdk5-Src interaction, lens epithelial cells were plated on fibronectin to initiate integrin-dependent cell adhesion. At subsequent times (0, 15min, 30min, 1hr, 2hr) cells were lysed for analysis of Cdk5 and Src, or stained with rhodamine-phalloidin to visualize the cytoskeleton. The results showed that Src was highly active for 30min-1hr after plating, and was then rapidly downregulated. As expected, the period of elevated Src activity was correlated with actin cytoskeleton reorganization, formation of lamellipodia, and the initial phase of cell spreading. As Src was downregulated, prominent stress fibers formed and spreading was augmented by extension of the lamellipodia between them. Downregulation of Src was also correlated with phosphorylation of Cdk5 at Y15, accompanied by an increase in Cdk5 activity, as shown by in vitro assay of Cdk5 kinase activity. To determine whether Cdk5 activation was causally related to stress fiber formation, cells were treated with Cdk5 specific inhibitor or transfected with dominant negative Cdk5 (dnCdk5) cDNA to inhibit Cdk5 activity. Spreading and stress fiber formation were not inhibited in cells treated with inhibitor or in cells transfected with dnCdk5. Moreover, inhibiting Cdk5 activity did not block downregulation of Src, implying that Cdk5 does not exert its effect on stress fiber formation by regulating Src activity. To determine whether the binding of Cdk5 to Src is necessary for Cdk5's effect on stress fibers, N/N1003a cells were transfected with a Cdk5 cDNA construct encoding the N-terminal binding site for Src. Expression of the corresponding peptide disrupted endogenous binding of Cdk5 to c-Src in lens epithelial cells and strongly inhibited both stress fiber assembly and cell spreading. These finding demonstrate that Cdk5 is recruited to sites of Src activity in spreading cells through a direct interaction between these proteins and required for the subsequent assembly of stress fibers.

POSTER 17

Lens placode formation and its regulation by BMP signaling

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The lens placode, a thickening of the head ectoderm, forms at embryonic day 9.5 (E9.5) of mouse development after contact with the optic vesicle. Placode formation was not associated with a higher rate of proliferation, but with higher cell death. This is consistent with the view of Hendrix and Zwaan, who postulated that adhesion to the optic vesicle limits spreading of prospective lens cells, leading to crowding and cell elongation. In conditional knockouts of the type I BMP receptor, Alk3, placode cells proliferated normally, but showed increased apoptosis. Placode cells lacking Alk2 proliferated more slowly than wild type, but had normal levels of apoptosis. Placodes lacking both Alk2 and Alk3 failed to form lenses. As in the single knockouts, they had increased apoptosis and decreased proliferation, compared to wild type. Double conditional knockout (DCKO) placodes were also thinner than normal and had reduced contact with the optic vesicle. Lens formation is Smad4 independent, as Smad4 knockout placodes formed lenses and had normal levels of cell death and proliferation. BMPs serve as survival factors and mitogens and are required for epithelial adhesion during lens induction.

POSTER 18

Inhibition of Cyclin Dependent Kinase 5 (Cdk5) Regulates E-Cadherin Trafficking and Degradation in Madin Darby Canine Kidney (MDCK) Epithelial Cells

Senthil S Saravanamuthu, Chun Gao, Peggy S Zelenka

Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health

E-cadherin is essential for epithelial cell polarity and cell-cell adhesion. Dynamic trafficking of E-cadherin to and from the cell surface modulates its levels within adherens junctions, thereby providing a mechanism for regulating cell adhesion. Previous work from this laboratory has shown that Cdk5, a protein kinase with known trafficking functions in neurons, is also involved in regulating cell-cell adhesion in epithelial cells of the lens and cornea. This work examines the role of Cdk5 in E-cadherin-dependent adhesion in MDCK cells, a widely used epithelial cell model. Inhibition of Cdk5 activity using 15uM olomoucine reduced E-cadherin based cell-cell adhesion by approximately 50%. To study the role of Cdk5 in E-cadherin trafficking and degradation, the surface localized E-cadherin in a confluent monolayer of cells was biotinylated using cell impermeable cleavable sulpho-NHS-SS biotin reagent. After 6h incubation in the presence or absence of olomoucine the cells were lysed and the remaining biotinylated E-cadherin was determined by affinity chromatography on neutravidin-coated beads followed by immunoblotting for E-cadherin. The total biotin-labeled E-cadherin remaining in the olomoucine-treated samples was less than that in the untreated controls (17% as compared to 47%, $p<0.001$). The inhibition of Cdk5 activity also increased the endocytosed internal pool of E-cadherin approximately 4-fold and decreased the surface membrane-associated pool by 14-fold as compared to the untreated control. Thus, Cdk5 activity affects both endocytotic recycling and degradation of E-cadherin to regulate epithelial cell-cell adhesion.

POSTER 19

Expression of mutated mouse myocilin induces open-angle glaucoma in transgenic mice

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We developed a genetic mouse model of open-angle glaucoma by expression of mutated mouse myocilin (Myoc) in transgenic (Tg) mice. The Tyr423His point mutation, corresponding to the severe glaucoma-causing Tyr437His mutation in the human MYOC gene, was introduced into BAC DNA encoding the full length mouse Myoc gene, and long flanking regions. Both wild-type (Wt) and Tg animals expressed Myoc in tissues of the irido-corneal angle and the sclera. Expression of mutated Myoc induced the accumulation of Myoc in cell cytoplasm and prevented its secretion into the extracellular space. The levels of ATPase-1 were reduced in the irido-corneal angle of Tg mice as compared with Wt animals. Tg mice demonstrated a moderate elevation of intraocular pressure (IOP), the loss of about 20% of the retinal ganglion cells (RGCs) in the peripheral retina, and axonal degeneration in the optic nerve. RGC depletion was associated with the shrinkage of their nuclei and DNA fragmentation in the peripheral retina. Pathological changes observed in the eyes of Tg mice are similar to those observed in glaucoma patients.

POSTER 20

Vitreous humor oxygen levels, oxygen consumption and human nuclear cataracts

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1. Ophthalmology & Visual Science, Washington University, St Louis, MO, 2. Barnes Retina Institute, St. Louis, MO.

Exposure to elevated oxygen and loss of the gel structure of the vitreous body are associated with the formation of nuclear cataracts. We measured oxygen in human eyes and tested the possibility that vitreous humor consumes oxygen. Oxygen levels were measured in patients undergoing vitrectomy using a fiber optic oxygen optode. Undiluted vitreous was obtained at vitrectomy and oxygen consumption was measured in vitro. Oxygen levels were normally very low (<1.5 mmHg) and increased greatly to ~10 mmHg immediately after surgery. Patients who had a prior vitrectomy had significantly higher vitreous oxygen levels than those undergoing their first vitrectomy. Samples from patients at the time of their initial vitrectomy (n=10) consumed oxygen at a mean rate of 1.4 μl·hr-1·ml-1. Vitreous fluid from patients undergoing a second vitrectomy (N=5) consumed oxygen at 0.5 μl·hr-1·ml-1 ($p < 0.01$). The increase in oxygen near the lens immediately after vitrectomy and for months after surgery is consistent with the hypothesis that increased exposure of the lens to oxygen causes nuclear cataracts. Human vitreous consumes oxygen, thereby helping to protect the lens from exposure to elevated oxygen. After vitrectomy, this protection is diminished.

POSTER 21

MFRP and the Regulation of Ocular Shape

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Nanophthalmos, a Greek term meaning "dwarf eye", is a rare inherited disorder characterized by a normal cornea, large lens, short axial length, and extreme hyperopia. We have recently identified mutations in the Membrane-type Frizzled-Related Protein gene at 11q23.3 as the cause of classic recessive nanophthalmos in an Amish-Mennonite kindred (PNAS 2005; vol 102: 9553-8). Patients homozygous for null mutations in MFRP can have fair to excellent vision with corrective lenses, but are at high risk of developing angle closure glaucoma and exudative retinal detachment.

MFRP is expressed at significant levels only in the eye, primarily in the retinal pigment epithelium. Most of the MFRP transmembrane protein extends outside the apical surface of the cell, where it is complexed with the trimeric short-chain collagen, C1QTNF5, and may interact with the retina. Expression begins early in development, prior to differentiation of retinal photoreceptors, suggesting that MFRP may be required during both embryonic and postnatal growth. Although the biochemical activities of MFRP protein are unknown, we eventually hope to understand what it does in the RPE, and how this tissue governs the development of ocular shape.

POSTER 22

Role of Kruppel-Like Transcription Factor KLF4 in the Post-natal Maturation and Maintenance of the Ocular Surface

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3. Department of Human Genetics and Molecular Medicine, Tel Aviv University, Israel. 4. Laboratory of Immunology, NEI, NIH, Bethesda MD.

Krüppel-like transcription factor KLF4 is one of the most highly expressed transcription factors in the mouse cornea (Invest. Ophthalmol. Vis. Sci. 45:429-440). We have deleted the Klf4 gene selectively in the cornea, conjunctiva and lens by mating Klf4-LoxP mice (Development 129:2619-28) with Le-Cre mice (Genes Dev. 14:2701-11). Klf4 conditional null (Klf4CN) mice displayed normal viability and fertility, rough ocular surface devoid of conjunctival goblet cells, hyperplastic iris and vacuolated anterior cortical fiber cells in the lens. Klf4CN cornea possessed fragile epithelium with swollen, vacuolated basal cells, edematous stroma and swollen, vacuolated endothelial cells. Excessive cell sloughing resulted in fewer cell layers (3-4 instead of 8-10) in spite of the increased cell proliferation in the Klf4CN corneal epithelium. Expression of keratin-12 and aquaporin-5 was downregulated, consistent with the Klf4CN corneal epithelial fragility and stromal edema, respectively. Microarray analysis identified a number of potential KLF4 target genes, whose expression was affected in the Klf4CN cornea. These observations show that KLF4 plays a critical role in post-natal maturation and maintenance of the ocular surface and suggest that the Klf4CN mouse can be tested as a model system for ocular surface pathologies such as dry eye, Meesmann's dystrophy, Fuchs dystrophy and Stevens-Johnson syndrome.

POSTER 23

The Cdk5 inhibitor olomoucine promotes corneal epithelial wound closure in vivo.

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1. LMDB/NEI/NIH, Bethesda, MD, 2. George Washington University, Washington, D.C.

The proline-directed kinase, Cdk5, regulates adhesion and migration of corneal epithelial cells in culture. Moreover, olomoucine, a Cdk5 inhibitor, accelerates corneal debridement wound closure in organ cultured eyes of normal mice. This study was undertaken to test the effect of olomoucine on corneal debridement wound closure in vivo in normal mice. A 1.5 mm central debridement wound was made in corneas of anesthesized mice. The treatment group received 20 µl of 15 µM olomoucine in phosphate-buffered saline (PBS) containing 0.1% dimethyl sulfoxide (DMSO), applied as drops to the wound area immediately after wounding (0 h) and again after 6 h. The control group received similar applications of PBS containing 0.1% DMSO. Eighteen hours after wounding mice were euthanized, eyes were removed, stained with Richardson's dye, and photographed. The remaining wound area was measured with image processing software. Images were coded during measurement to prevent experimenter bias. Measurements of 28-30 mice in each group indicated that the remaining wound area in the control group was approximately 2-fold greater than that of the olomoucine treated mice ($p<0.001$). Two weeks after wounding, restratification of the cornea was identical in both olomoucine treated and control groups and neither group showed evidence of inflammation or stromal disorganization. These findings indicate that Cdk5 inhibitors promote corneal epithelial cell migration in vivo as in vitro and suggest that this class of compounds may be therapeutically useful for treatment of corneal epithelial erosions.

POSTER 24

Serial Analysis of Gene Expression (SAGE) in the Zebrafish Lens and Cornea

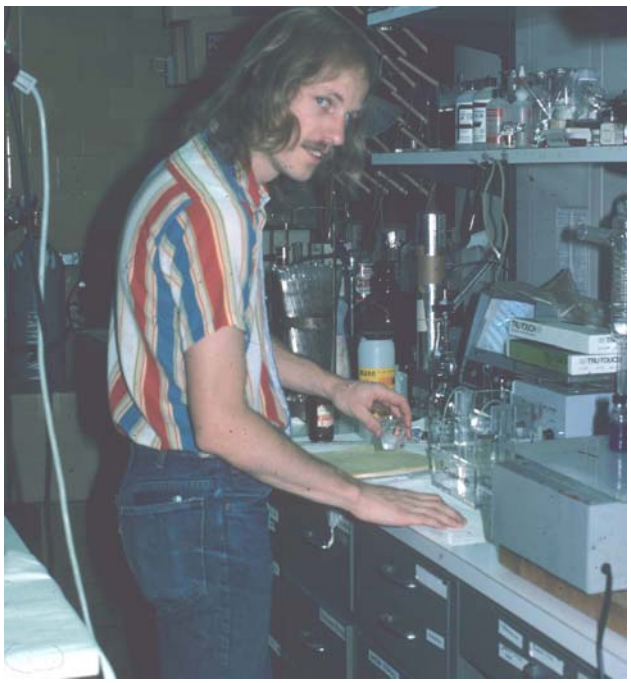
Hagit Ulanovsky[1,2], Barbara Norman[1] and Joram Piatigorsky[1]

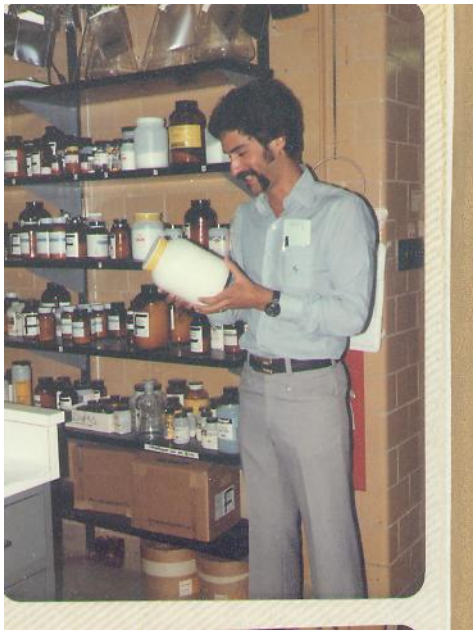
1. LMDB, NEI 2. International Graduate Center of Evolution, Institute of Evolution, University of Haifa, Israel

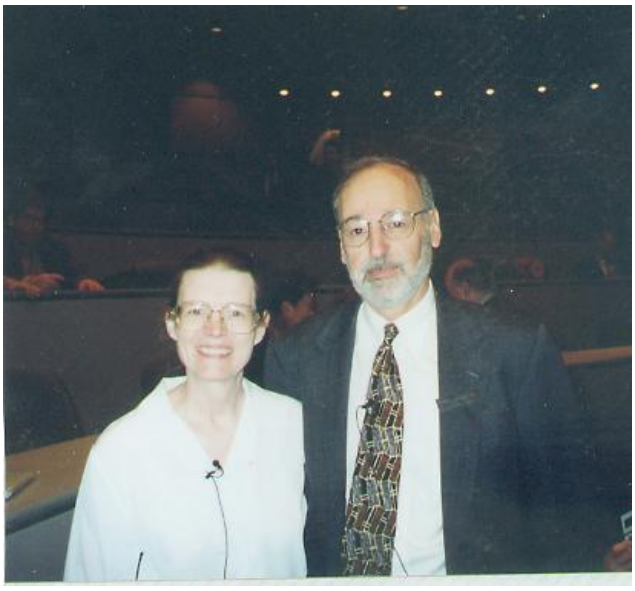
PURPOSE: To provide a detailed gene expression profile of the normal zebrafish (Danio rerio) lens and cornea.

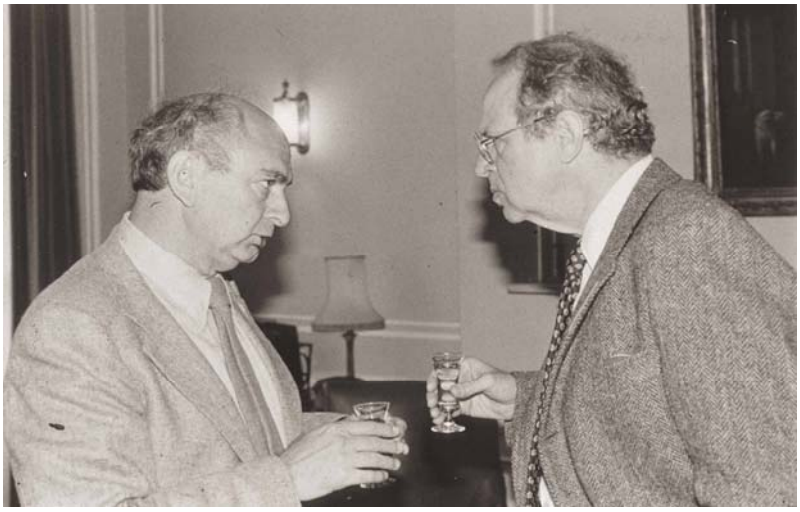
METHODS: Serial analysis of gene expression (SAGE) was performed on adult zebrafish lens and cornea.

RESULTS: The SAGE library of the zebrafish lens consisted of a few thousand tags. As expected, the most abundant expressed genes encoded crystallins, especially γS1-crystallin. A relatively large amount of duplicated ditags were created in the lens library due to the great enrichment of crystallin mRNAs in this tissue. A SAGE library of adult zebrafish cornea is presently being constructed. We expect to find tags for gelsolin-like protein 1 (formerly called C/L-gelsolin) to be highly abundant in the cornea SAGE library. Special interest attaches to the nature of zebrafish transcription factors in the corneal SAGE library in order to guide our studies on gene expression of the corneal crystallin, gelsolin-like protein 1. We anticipate that comparison of corneal SAGE tags derived from zebrafish, mice and rats will provide insights on corneal function and evolution.







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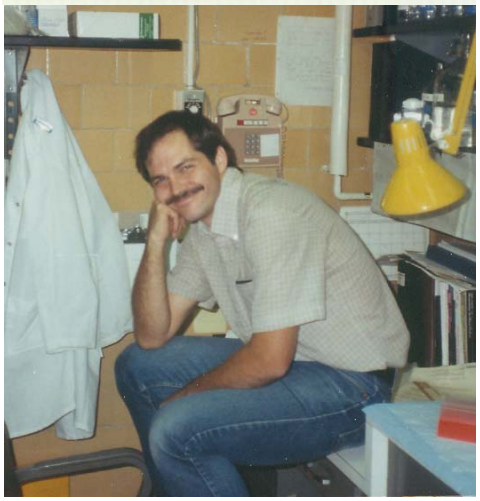


CONSERVATION OF INTRONS


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THE DUCK δ-CRYSTALLIN GENE: CONSERVATION OF INTRONS


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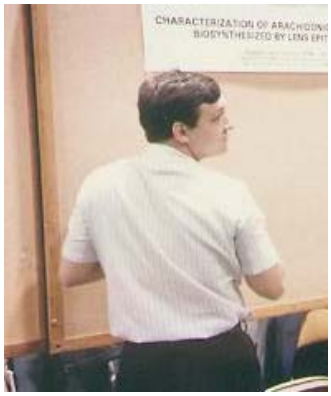

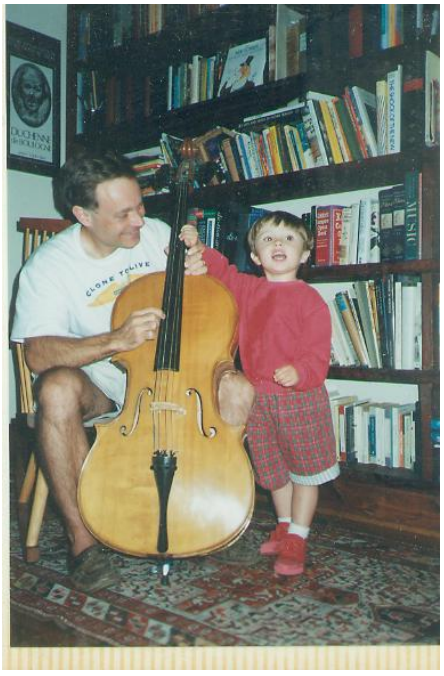


Landau


50
REGULATION OF αB-CRYSTALLIN EXPRESS


Gone but not
forgotten
Good luck
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2006 LMDB Anniversary Symposium

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Special Thanks…to Christine Crawford and Kimberly White for their excellent administrative support and help in preparing the photographs for this book.

Special Thanks…to Belinda Davis (Office of the Scientific Director, NEI) for her tremendous help in organizing this meeting. The members of the LMDB are truly grateful to her friendly, invaluable role in making the LMDB 25th Anniversary Symposium a reality.

LMDB is grateful for the many years of support from the National Eye Institute and for sponsoring this symposium.

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