3rd Retinal Cell And Gene Therapy Innovation Summit

This book discusses applications of pluripotent stem cells to study eye disease in vitro and to create novel therapies for degenerative eye diseases. Chapters are contributed by experts in the field and cover such topics as the use of pluripotent stem cells in 2D and 3D engineering of ocular tissues for disease modelling and drug testing as well as approaches to replace degenerated RPE and photoreceptors in macular degeneration and retinitis pigmentosa. Pluripotent Stem Cells in Eye Disease Therapy presents a comprehensive discussion of basic science and clinical applications and is an indispensable resource for everyone from advanced graduate students to advanced professionals who want to learn about the potential of stem cell biology and its role in the field of retinal diseases.

A discussion of all the key issues in the use of human pluripotent stem cells for treating degenerative diseases or for replacing tissues lost from trauma. On the practical side, the topics range from the problems of deriving human embryonic stem cells and driving their differentiation along specific lineages, regulating their development into mature cells, and bringing stem cell therapy to clinical trials. Regulatory issues are addressed in discussions of the ethical debate surrounding the derivation of human embryonic

stem cells and the current policies governing their use in the United States and abroad, including the rules and conditions regulating federal funding and questions of intellectual property.

Regenerative medicine – stem cell and gene-based therapy – offers a new approach for restoring function of damaged organs and tissues. This is the first book to cover the major new aspects and field of regenerative medicine. This title is therefore a timely addition to the literature. It brings together the major approaches to regenerative medicine in one text, which ensures that techniques learnt in one discipline are disseminated across other areas of medicine.

In 1984, we organized a two-day symposium on retinal degenerations as part of the biennial meeting of the VI International Society for Eye Research, held in Alicante, Spain. The success of this first meeting led to the second held, two years later in Sendai, Japan, organized as a satellite of the VII ISER. We were fortunate that these meetings began at a time of vigorous research activity in the area of retinal degenerations, thanks to the financial support of the Retinitis Pigmentosa Foundation and the strong encouragement of its scientific director, Dr. Alan Laties. Significant advances were made so that every two years scientists were eager to meet to share their findings. The programs included presentations by both basic and clinical researchers with ample time for informal discussions in a relaxed atmos phere. Many investigators met for the first time at these symposia and a number of fruitful

collaborations were established. This book contains the proceedings of the VI International Symposium on Retinal Degenerations held November 6-10, 1994, in Jerusalem. As with the other meetings, some new areas were covered. One session was devoted to apoptosis, an important process involved in cell death in inherited retinal degenerations. Another session was on invertebrate photoreceptors, where numerous mutations have now been identified that lead to altered function or degeneration of the retina. All participants were invited to submit chapters and most complied. We thank them for their contributions.

Updated throughout to reflect the latest discoveries in this fast-paced field, this Sixth Edition, provides an accessible, student-friendly introduction to modern genetics. Designed for the shorter, less comprehensive course, the Sixth Edition presents carefully chosen topics that provide a solid foundation to the basic understanding of gene mutation, expression, and regulation. It goes on to discuss the development and progression of genetics as a field of study within a societal and historical context. The Sixth Edition includes new learning objectives within each chapter which helps students identify what they should know as a result of their studying and highlights the skills they should acquire through various practice problems.

With almost twice as many chapters, this new edition of Pediatric Retina now includes important information on the development of the eye and retina, basic/translational science of retinal diseases in infants and children, telemedicine using wide-angle

imaging for diagnosis and longitudinal management of infants and children, as well as international approaches to care with focus on retinopathy of prematurity.
Glaucoma is a neurodegenerative disease that can lead to a complete loss of vision due to retinal ganglion cell (RGC) death. Therapies that have the capacity to protect and rescue stressed RGCs remain a critical unmet need in glaucoma management. Neurotrophic factor (NF) gene therapy is a promising therapeutic approach that can address this current clinical deficiency by providing damaged RGCs with extrinsic neurotrophic support as a means of protection and repair. Moreover, a non-viral approach to the delivery of NF-encoding plasmid DNA (pDNA) confers many advantages over a viral approach for its improved immunogenicity and mutagenesis risks, patient compliance and large-scale manufacturing cost and feasibility. In this research, the main objective was to address the challenges facing non-viral NF gene therapy field for the retina, through development of three in vitro model systems that aim to facilitate the preclinical screening and identification of promising NF gene delivery systems. The first model system developed was a versatile co-culture model that simulates cellular interactions between "healthy" and "stressed" cells in the retina. Furthermore, through incorporation of techniques including enzyme-linked immunosorbent assay (ELISA), immunofluorescent imaging, and neurite tracing into the co-culture setup, the model system enables a systematic evaluation of the therapeutic potential of gene delivery systems through assessment of bioavailability and bioactivity

of therapeutic proteins produced from transfected cells. The second model system was a new potential RGC cell line, termed XFC series of cells, that express key RGC characteristics and suitable for the evaluation of RGC-aimed gene therapies. Derived from multipotent retinal stem cells (RSCs), XFC cells express multiple RGC markers including Map-2, Rbpms, and Tubb3, and exhibit RGC-like neurite extension capacity in response to brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and rho-kinase inhibitor (RKI) Y-27632 activation. The feasibility of the cell model was further validated in the described co-culture setup as XFC cells were able to validate the bioactivity of the BDNF proteins released by transfected cells. The third model system developed was a stem cell-derived 3D "mini-retina" culture model (termed MiEye series of retinal neurospheres) that contains multiple retinal cell types and enables in vivo-like gene delivery assessment. Derived from differentiating multipotent RSCs in 3D culture, MiEye retinal neurospheres with different retinal biomarker expressions can be generated using different protocols. Moreover, by harnessing the tissue-like arrangement of retinal cells in MiEye retinal neurospheres, it enables the assessment of infiltration and transfection capacity of gene delivery systems in tissue-like structure, towards the establishment of a more representative in vitro-in vivo correlation and prediction of in vivo gene delivery feasibility. Concurrent to model system development, aspects that focus on the development of non-viral gene delivery systems for the retina were also explored. The first aspect involved the

optimization of gemini surfactant (GS) lipid nanoparticle systems (GL-NPs) physicochemical properties by evaluating the roles of minicircle plasmid (MC), sonication processing, and total NP component concentration (TNPC). Through dynamic light scattering and fluorescent correlation spectroscopy physicochemical characterizations, it was found that the size, particle size distribution, and zeta potential could be effectively optimized through sonication processing and TNPC. Moreover, the number of pDNA per particle homogeneity can be improved by formulating GL-NPs with MCs. The second aspect involved an investigation on the application of carbon nanotubes (CNTs) as a gene delivery vehicle to retinal cell types. More specifically, GS-functionalized SWNT gene delivery system (*f*-ptSWNT) was developed and demonstrated the ability to deliver pDNA to a retinal astrocyte cell line. The results demonstrate the feasibility of utilizing *f*-ptSWNT to deliver pDNA to retinal cells and serves as a starting point for future *f*-ptSWNT retinal gene delivery system development. The development of the three in vitro model systems in this thesis collectively aims to facilitate the preclinical screening and development of non-viral NF gene therapies in a synergistic manner, covering key areas of assessments that are critical to in vivo therapeutic success. Furthermore, concurrent developments in non-viral gene delivery systems through GL-NP optimization and CNT exploration also advance the knowledge towards the development of better non-viral gene delivery systems.

The mammalian retina contains over sixty diverse cell types, which are grouped into seven major cell classes: rod photoreceptors, cone photoreceptors, bipolar interneurons, amacrine interneurons, horizontal interneurons, retinal ganglion cells (RGCs), and Muller glia. During development, all cell classes arise from a common pool of retinal progenitor cells (RPCs), in a highly conserved and partially overlapping sequence. RPCs simultaneously regulate the growth of the retina by undergoing continuous rounds of cell division. Therefore, a complex network of extrinsic signals and intrinsic transcription factors is required to maintain the balance between cell cycle progression and neuronal (or glial) differentiation. Proneural basic helix-loop-helix (bHLH) factors coordinate multiple elements of retinal neurogenesis. Although Notch regulation of bHLH genes is an evolutionarily conserved module, the tissue-specific mechanisms are incompletely defined. In the developing mouse retina, Atoh7 regulates RGC competence and Neurog2 is required for the progression of neurogenesis. These two transcription factors are extensively coexpressed in RPCs, thereby suggesting a similar mode of regulation. In Chapter 2, we directly compared Atoh7 and Neurog2 expression at the earliest stages of retinal neurogenesis, in a broad spectrum of Notch pathway mutants. Here, a Notch1, Rbpj, and Hes1-mediated signal represses Atoh7. Yet, the combined activities of Notch1, Notch3, and Rbpj regulate Neurog2 patterning, independent of Hes1, Hes3, or Hes5. Finally, we tested Notch regulation of Jag1 and Pax6, to establish the proper context for distal Neurog2 patterning. We found that Rbpj

blocks coexpression of Jag1 and Neurog2 most-distally, while stimulating Pax6 in an adjacent domain. Together, our data suggest that Notch signaling controls the overall tempo of retinal neurogenesis, by integrating cell fate specification with the developmental status of cells ahead of this wave. Although Neurog2 is required for the overall temporal progression of neurogenesis, the molecular mechanism behind this regulation remains elusive. Additionally, it was previously shown that ectopic Ascl1, another bHLH, could rescue the delay of RGC differentiation seen in Neurog2 mutants. Combined, this suggests that Neurog2 and Ascl1 regulate similar processes, with respect to neuronal wave propagation. In Chapter 3, we directly compared the average RPC cell cycle length between Neurog2GFP/+, Neurog2GFP/GFP, and Neurog2GFP/Ascl1 retinas. Here, we found that Neurog2 is required for normal cell cycle exit, whereby ectopic Ascl1 only partially rescues the mutant defects. RNA-seq analysis for all three genotypes demonstrated that genes associated with RGC differentiation and cell cycle progression were significantly downregulated in Neurog2 mutants, compared to controls. Interestingly, ectopic Ascl1 only rescued one RGC gene, at the expense of all others. Combined, this data implies that although ectopic Ascl1 restores the wave of neurogenesis, it does not completely substitute for Neurog2 at the same molecular level. Therefore, each factor must target a unique subset of downstream genes, in addition to common genes involved in general neurogenesis. Authors Dave Nelson and Mike Cox combine the best of the laboratory and best of the

classroom, introducing exciting new developments while communicating basic principles of biochemistry.

Virtually any disease that results from malfunctioning, damaged, or failing tissues may be potentially cured through regenerative medicine therapies, by either regenerating the damaged tissues in vivo, or by growing the tissues and organs in vitro and implanting them into the patient. Principles of Regenerative Medicine discusses the latest advances in technology and medicine for replacing tissues and organs damaged by disease and of developing therapies for previously untreatable conditions, such as diabetes, heart disease, liver disease, and renal failure. Key for all researchers and instituions in Stem Cell Biology, Bioengineering, and Developmental Biology The first of its kind to offer an advanced understanding of the latest technologies in regenerative medicine New discoveries from leading researchers on restoration of diseased tissues and organs

Every new copy includes access to the student companion website Updated throughout to reflect the latest discoveries in this fast-paced field, Essential Genetics: A Genomics Perspective, Sixth Edition, provides an accessible, student-friendly introduction to modern genetics. Designed for the shorter, less comprehensive course, the Sixth Edition presents carefully chosen topics that provide a solid foundation to the basic understanding of gene mutation, expression, and regulation. It goes on to discuss the development and progression of genetics as a field of study within a societal and

historical context. The Sixth Edition includes new learning objectives within each chapter which helps students identify what they should know as a result of their studying and highlights the skills they should acquire through various practice problems. What's new in the Sixth Edition? Chapter 1 includes a new section on the origin of life Chapter 2 includes a revised discussion of the complementation test and how it is used to determine whether two mutations have defects in the same gene Chapter 3 incorporates new data showing that the folding of interphase chromatin into chromosome territories has the form of a fractal globule. It also includes a new section on progenitor cells and embryonic stem cells Chapter 4 includes a new section discussing how copy-number variation in human amylase evolved in response to increased dietary starch as well as the latest on hotspots of recombination Chapter 5 is updated with the latest information on hazards of polycarbonate food containers. It also includes a new section on the genetics of schizophrenia and autism spectrum disorder Chapter 6 includes a revised section on restriction mapping and also discusses the newest massively parallel DNA sequencing technologies that can yield the equivalent of 200 human genomes' worth of DNA sequence in a single sequencing run Chapter 7 has been updated with a shortened and streamlined discussion of recombination in bacteriophage Chapter 8 includes new discoveries concerning the mechanisms of intrinsic transcriptional termination as well as rho-dependent termination Chapter 9 is updated with a new section on stochastic effects on gene expression and an expanded

discussion of the lactose operon. There is also a revised discussion of galactose gene regulation in yeast, as well as new sections on lon noncoding RNAs Chapter 10 includes new sections on ancient DNA sequences of the Neandertal and Denisovan genomes Chapter 11 examines master control genes in development Chapter 12 includes a new section on the repair of double-stranded breaks in DNA by nonhomologous end joining or template-directed gap repair Chapter 13 has been extensively revised with the latest data on cancer. Chapter 14 includes a new section on the detection of natural selection, as well as a new section on conservation genetics Key Features of Essential Genetics, Sixth Edition: New Learning Objectives within each

Engineered Adeno-Associated Viral Vectors for Gene Therapy in the Retina

Retinal development involves defined guidance of neuronal axons to their destined cells through cell adhesion molecules, by compilation of cell-cell connections at synapses. Cell adhesion molecules promote oriented axonal outgrowth and help in target synaptic specificity to maintain histoarchitecture of retina. Cell adhesion molecules like cadherins are the products of expression of long genes>200 kilobases of genomic DNA. Topoisomerase II beta (Top2b) involved in modulating DNA supercoiling by catalyzing the double strand breaks and passing the stands through one another. Top2b is pervasively expressed in all terminally differentiated cells and participates in gene transcription. Recent studies have shown that Top2b facilitates the expression of long genes. However, its particular role in transcription of cadherins and effect on

organization of retinal cells such as retinal ganglion cells and horizontal cells remain unclear. In current study, the role of Top2b in expression of cadherin genes was analyzed in iii developing chick retina via immunohistochemistry and real-time PCR. Top2b function in developing retina was inhibited by injections of 500æM Top2b catalytic inhibitor (ICRF-193) into sub-retinal space at embryonic day 4 (E4). Retinal tissues were analyzed by immunohistochemistry using cell specific markers, e.g., Brn3a for ganglion cells, 4F2 for horizontal cells, and Tuj1 for neuronal processes at E6, E8, E10, and E12. We showed that inhibition of Top2b by ICRF-193 i) reduced the expression level of cadherin genes, e.g., N-cadherin (Cdh2), Cadherin-6B (Cad6B), Cadherin-7 (Cdh7), and Cadhrin-8 (Cdh8)in developing retina; ii)led to a disoriented cellular organization; and iii) delayed migration of RGCs and HCs. The results from immunohistochemistry were confirmed by quantitative real-time PCR (qRT-PCR). Results from qRT-PCR showed significant reduction in expression levels of cadherin genes, e.g., Cad6B and Cdh7 with an average fold reduction of 2.5 prominent at embryonic day 10 (E10) (p

Neuronal plasticity is present in the early postnatal mouse visual system and is used as a model for nervous system development. The dorsal lateral geniculate nucleus (dLGN) of mice have intermingled binocular retinal ganglion cell (RGC) afferent projections that develop in to eye specific regions during the first postnatal week. Development of eye specific regions occur through a competitive process as erroneous RGC inputs in an

eye specific dLGN region are pruned and replaced by RGC inputs from the appropriate eye. The driver for the development of eye specific regions in the dLGN remains an area of debate and studies have focused on the role patterned electrical activity in the retina and molecular cues play in this process. The work presented contributes to the knowledge on development of eye specific regions in the mouse by testing these hypotheses in addition to a third hypothesis: non-retinal inputs play a role in the segregation of retino-geniculate projections. The presence of cholinergic input from the parabrachial region of the brainstem to the dLGN was studied to determine if this input is present during the development of eye specific regions and is presented in Chapter 1. I found that this non-retinal input is not present at the time of eye specific region development, indicating that this input is not likely to play a role in development of eye specific regions. Chapter 2 studied the role that patterned retinal activity plays in the development of eye specific regions by using the mutant mouse lacking a functional [beta]2 subunit of the nicotinic acetylcholine receptor subunit ([beta]2-/- nAChR). All mutant mice and control mice expressed retinal waves of coordinated electrical retinal activity, but [beta]2-/- nAChR mice displayed abnormal eye specific region development, indicating that retinal waves are not sufficient to drive normal eye specific region development. Chapter 3 studied the role that molecular cues play in the development of eye specific regions by undergoing a microarray study to determine if gene expression in the retina and dLGN differ between control and [beta]2-/- nAChR

mice. Data showed there is a subset of genes that are differentially expressed in a temporal and tissue specific manner, potentially implicating an altered expression of non-[beta]2 nAChR associated genes in the altered development reported in the [beta]2-/- nAChR mice. Chapter 4 studied the role that patterned and spontaneous retinal activity play in development by using a pharmacological tool that significantly alters retinal activity in developing mice. All mice that were treated with the pharmacological drug continued to express normal development of eye specific regions in the dLGN, indicating that expression of normal retinal activity is not essential for the development of these regions. The results presented demonstrate that non-retinal cholinergic input and postnatal electrical retinal activity are not essential for the development of a normal retino-geniculate projection pattern. However, results indicate that an altered molecular expression of genes in the dLGN and retina of [beta]2-/- nAChR mice is a potential source for the altered eye specific region development expressed in these mice. These findings contrast previous studies that concluded retinal activity to have an instructive role in visual system development, meanwhile, supporting studies that found expression of molecular cues to be essential for normal visual system development. Overall, these results indicate that electrical activity is playing a minor role, and molecular cue expression is likely to play a primary role, in the development of the mouse visual system.

This Atlas of Inherited Retinal Disorders provides a thorough overview of various

inherited retinal dystrophies with emphasis on phenotype characteristics and how they relate to the most frequently encountered genes. It also meets the previously unmet needs of PhD students who will benefit from seeing the phenotypes of genes they work on and study. Further, because genetic-testing costs are quite high and spiraling higher, this Atlas will help geneticists familiarize themselves with the candidate gene approach to test patients' genomes, enabling more cost-efficient testing. This invaluable atlas is organized into eight sections starting with an introduction to the basic knowledge on retinal imaging, followed by diseases listed according to inheritance pattern and disorders with extraocular manifestations grouped by defining features. This structure will be intuitive to clinicians and students studying inherited retinal disorders.

Müller cells may be used in the future for novel therapeutic strategies to protect neurons against apoptosis (for example, somatic gene therapy), or to differentiate retinal neurons from Müller/stem cells. Meanwhile, a proper understanding of the gliotic responses of Müller cells in the diseased retina, and of their protective vs. detrimental effects, is essential for the development of efficient therapeutic strategies that use and stimulate the neuron-supportive/-protective - and prevent the destructive - mechanisms of gliosis.

?This book provides the ophthalmologist with the most recently available data on the macular dystrophies, a group of many different inherited or sporadic eye conditions

linked by a problem with photoreceptors or other structures of the central retina. Internationally recognized experts in the field present the latest evidence and discuss their own personal experiences with regard to each of the principal dystrophies as well as some very rare entities. Topics covered include molecular biology, state-of-the-art diagnostic techniques, and the newest treatment options, including still experimental therapies. Attention is also devoted to a range of issues that continue to be debated. The editors have taken care to ensure that chapters are of a uniformly high standard while not sacrificing the originality of the individual authors. Macular Dystrophies will fully acquaint the reader with both the latest research findings and the current and emerging approaches to diagnosis and treatment.

This book provides a series of comprehensive views on various important aspects of vertebrate photoreceptors. The vertebrate retina is a tissue that provides unique experimental advantages to neuroscientists. Photoreceptor neurons are abundant in this tissue and they are readily identifiable and easily isolated. These features make them an outstanding model for studying neuronal mechanisms of signal transduction, adaptation, synaptic transmission, development, differentiation, diseases and regeneration. Thanks to recent advances in genetic analysis, it also is possible to link biochemical and physiological investigations to understand the molecular mechanisms of vertebrate photoreceptors within a functioning retina in a living animal. Photoreceptors are the most deeply studied sensory receptor cells, but readers will find

that many important questions remain. We still do not know how photoreceptors, visual pigments and their signaling pathways evolved, how they were generated and how they are maintained. This book will make clear what is known and what is not known. The chapters are selected from fields of studies that have contributed to a broad understanding of the birth, development, structure, function and death of photoreceptor neurons. The underlying common word in all of the chapters that is used to describe these mechanisms is “molecule”. Only with this word can we understand how these highly specific neurons function and survive. It is challenging for even the foremost researchers to cover all aspects of the subject. Understanding photoreceptors from several different points of view that share a molecular perspective will provide readers with a useful interdisciplinary perspective.

Retinitis pigmentosa (RP) is a rare disease responsible for the majority of the cases ofhereditary blindness. RP is a genetically heterogeneous disease caused by more than 3000 mutations in over 60 genes without an effective treatment. Irrespectively of the causative mutation, there are common traits to RP: photoreceptor cell death and retinal inflammation. In this thesis we have studied three potential mutation-independent therapeutic strategies for RP treatment. The insulin receptor, a key controller of metabolism, also regulates neuronal survival and synaptic formation, maintenance, and activity. Here we present evidence linking impaired insulin receptor signaling with RP. In physiological conditions, insulin receptor is expressed inall the

retinal layers depicting prominent levels in horizontal and ganglion cells fibers. In the rd 10retinas, a mouse model of RP, the insulin receptor and its down stream effector phospho-S6were selectively decreased in the horizontal cells. In parallel, we observed aberrant synapses between rod photoreceptors and the post-synaptic terminals of horizontal and bipolar cells. A gene therapy strategy to induce sustained proinsulin production restored retinal insulin receptor signaling, increasing S6 phosphorylation... Inherited retinal degenerations are genetically heterogeneous conditions affecting roughly 1:3000 people and are characterized by the loss of photoreceptors. Progressive retinal degenerative disease is the leading cause of vision loss in industrialized countries, and is the result of a wide range of mutations, mostly in rod-specific transcripts. Over 140 disease-causing genes have been identified to date. As the genetic mechanisms underlying inherited forms of retinal degeneration are identified, gene therapy is becoming a promising approach for the treatment of many inherited blinding diseases. Indeed, the recent success of three clinical trials using adeno-associated virus (AAV) to deliver a normal copy of the RPE65 gene to the retinas of Leber congenital amaurosis (LCA) patients illustrates the potential of gene therapy in the retina. AAV has been shown safe and effective especially in a younger cohort of patients. Some important obstacles remain, however, for AAV-mediated gene therapy to become widely applicable across the range of existing retinal degenerative diseases. It will be essential to carefully evaluate the method used to deliver therapeutic genetic

material to the retina, as this will determine the success of the treatment. The serotype of vector used, the promoter chosen to drive expression and the method of injection are important components of the gene delivery system. A wide variety of AAV serotypes exist with different tropisms for cell populations in the retina, potentially allowing treatments to be targeted to specific cell types. The retinal cell types AAV can infect differs, however, depending on whether the vector is delivered into the vitreous cavity or the subretinal space. Subretinal injections, which were used in the LCA trials, result in the creation of a retinal detachment and localized injury to the retina while delivering high concentrations of transgene to only a limited area. An intravitreal approach has the potential to transduce panretinally and is less invasive, and therefore preferable, but naturally occurring serotypes of AAV transduce photoreceptors poorly from the vitreous, as a result of structural barriers that exist on the inner surface of the retina. Recent advances in the understanding of AAV and the production of viral vectors have shown the flexibility of this virus, indicating that its function can be altered and tailored to the requirements of retinal gene therapy. A directed evolution approach has been used to select, out of a highly diverse library of AAV capsid variants, a novel variant with improved tropism for Müller glia. And in a parallel approach, residues on the capsid surface have been mutated to avoid ubiquitination and altering the nuclear trafficking of the virus. This dissertation examines the use of engineered viral vectors for gene therapy in the retina. The creation of a novel variant of AAV, called 7m8, which is

characterized by increased transduction of photoreceptors from the vitreous, is described below. 7m8 was derived from an AAV2 peptide insertion library and contains a 7mer motif. Injected intravitreally, 7m8 transduces cells throughout the retina, including photoreceptors in the outer retina, significantly more efficiently compared to the parental serotype. Expression was restricted to photoreceptors using a rhodopsin promoter. This virus, as well as the previously described Müller-specific variant ShH10, was used to deliver a wild-type copy of the retinoschisin gene to mice lacking this protein. Retinoschisin is secreted from photoreceptors, and retinas deficient in this protein are severely structurally impaired. Subretinal injections, which are damaging in nature, are therefore suboptimal because they are likely to cause additional injury. We show that 7m8 is able to efficiently target photoreceptors via intravitreal injection in this mouse model, leading to high levels of retinoschisin protein production, as well as structural and functional rescue. This rescue is longer lasting than that seen using ShH10, indicating the importance of targeting photoreceptors in this disease model. AAV9 has been shown to transduce the murine retina when injected intravenously through the tail vein. We used two surface tyrosine-to-phenylalanine mutations to improve the retinal expression of AAV9, and demonstrated that these mutations lead to higher infectivity of all retinal layers, most dramatically in photoreceptors and the inner nuclear layer, but also including the retinal pigment epithelium and ganglion cells. This novel vector was then used to explore the bifunctionality of the Nxnl1 gene, which

encodes two isoforms of the rod-derived cone viability factor (RdCVF). The short form of RdCVF is secreted and has been shown to support cone survival, while the long isoform is retained intracellularly and has been implicated in redox signaling. AAV92YF and 7m8 were used to express the two isoforms of RdCVF in the rd10 mouse model of retinitis pigmentosa. RdCVF rescued cone survival when injected intravenously or intravitreally, but had little effect on rod survival. Early expression of RdCVFL in dark-reared rd10 mice delayed rod, and subsequently cone death.

Pituitary Adenylate Cyclase-Activating Polypeptide is the first volume to be written on the neuropeptide PACAP. It covers all domains of PACAP from molecular and cellular aspects to physiological activities and promises for new therapeutic strategies. Pituitary Adenylate Cyclase-Activating Polypeptide is the twentieth volume published in the Endocrine Updates book series under the Series Editorship of Shlomo Melmed, MD.

This book provides an overview of the types, sources, and applications of stem cells in regenerating various ocular tissues, with a perspective on both potential applications of stem cells and possible challenges. The scope of the chapters include both preclinical and clinical applications, including stem cell-derived therapies based on endogenous tissue repair; stem cell transplantation and cell replacement therapy; gene therapy; and in vitro disease modelling. Additionally, the volume presents applications in both anterior and posterior ocular disease, with a particular focus on diseases of the ocular surface, cornea, limbus, and retina, including inherited retinal dystrophies as well as

acquired diseases, such as age-related macular degeneration. Regenerative Medicine and Stem Cell Therapy for the Eye is an ideal book for advanced researchers in stem cell and ocular biology as well as clinical ophthalmologists, and will be of interest to readers with backgrounds in developmental biology and bioengineering. This book also Skillfully reviews cutting-edge advances in stem cell biology as applied to regenerative medicine and ocular disease Provides expert viewpoints on key hurdles and challenges to successful implementation of stem cell-derived therapies in the clinical domain Offers a multi-disciplinary, broad understanding of cell-based therapies for ocular diseases by incorporating perspectives from biomedical scientists, physicians, and engineers Examines the connection between cell therapy and gene editing, in particular relation to ocular disease

This book discusses why specific diseases are being targeted for cell-based retinal therapy, what evidence exists that justifies optimism for this approach, and what challenges must be managed in order to bring this technology from the laboratory into routine clinical practice. There are a number of unanswered questions (e.g., surgical approach to cell delivery, management of immune response, optimum cell type to transplant) that very likely are not going to be answered until human trials are undertaken, but there is a certain amount of “de-risking” that can be done with preclinical experimentation. This book is essential reading for scientists, clinicians, and advanced students in stem cell research, cell biology, and ophthalmology.

Abstract Development and Assessment of Gene Therapies for Inherited Blinding Diseases By Kathleen Durgin Kolstad Doctor of Philosophy in Molecular and Cell Biology University of California, Berkeley Professor John Flannery, Committee Chair There are two therapeutic approaches for inherited retinal disease addressed in this dissertation: we sought to slow retinal degeneration and reverse visual loss after complete photoreceptor apoptosis. In the first approach, by viral gene transfer to the support cells of the retina, Müller glia (RMCs), we achieved sustained secretion of human glial derived neurotrophic factor (hGDNF) (Chapter 3). We hypothesized that hGDNF production by retinal glia will enhance the protective affects of RMCs in the diseased retina and help slow photoreceptor degeneration. Furthermore, this method avoids extra photoreceptor stress caused by direct hGDNF gene transfer to cells that are already stressed. We were able to optimize gene transfer to RMCs and observe the beginnings of functional rescue in an animal model of autosomal dominant retinitis pigmentosa with this technique. One major advantage of this therapeutic approach is that it is applicable to multiple retinal disease genotypes. The second approach to ocular gene therapy presented in this dissertation was to re-introduce photosensitivity to the retina after complete photoreceptor degeneration (Chapter 4). To this end, we employed the engineered light activated glutamate receptor (LiGluR) to confer light sensitivity on retinal ganglion cells (RGCs) in the diseased retina. We first showed LiGluR mediated RGC photo-activation in in vitro retinal tissue preparation. We then

characterized in vivo cell population responses (visually evoked potentials, VEPs) in V1 when retinal input was limited to LiGluR induced activity in the retina. VEPs driven by LiGluR are approximately 50% of the amplitude of full field light flash driven responses in the wild type animal. LiGluR driven cortical responses in blind animals suggest that it is a promising therapy for restoring visual function and processing in the late stages of retinal degeneration. In the third part of this dissertation (Chapter 2a and 2b), the goal was to develop and assess methods of making ocular gene therapies safer and more efficacious. Current gene therapies for retinal degenerative diseases rely on subretinal delivery of viral vectors carrying therapeutic DNA. However, this method of delivery limits the viral transduction profile to the region of injection and seriously compromises the retina during detachment. We have identified natural barriers to viral vector delivery to the outer retina from the vitreous. Furthermore, we have developed artificial methods and characterized disease states that allow these barriers to be overcome. The understanding of and the ability to manipulate barriers to vitreal delivery of viral vectors will help avoid the limitations, risks, and damage associated with subretinal injections. This book presents new and noteworthy research into retinal diseases. It focuses on what we currently know about the environment, genetics and mechanisms that lead to retinal degenerations, new diagnostics, and innovative therapeutic modalities to preserve vision. Written by renowned scientific investigators, this innovative collection of treatment strategies and technological discoveries allows for the realistic translation

of research into practice.

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH): The Quintessential Moonlighting Protein in Normal Cell Function and in Human Disease examines the biochemical protein interactions of the multi-dimensional protein GAPDH, further considering the regulatory mechanisms through which cells control their functional diversity. This protein's diverse activities range from nuclear tRNA export and the maintenance of genomic integrity, to cytoplasmic post-transcriptional control of gene expression and receptor mediated cell signaling, to membrane facilitation of iron metabolism, trafficking and fusion. This book will be of great interest to basic scientists, clinicians and students, including molecular and cell biologists, immunologists, pathologists and clinical researchers who are interested in the biochemistry of GAPDH in health and disease. Contextualizes how GAPDH is utilized by cells in vivo Provides detailed insight into GAPDH post-translational modifications, including functional diversity and its subcellular localization Includes forward-thinking exposition on tough topics, such as the exploration of how GAPDG performs functions, how it decides where it should be present and requisite structural requirements

Sight loss and blindness is a very prevalent cause of disability. Retinal diseases leading to visual loss affect many people worldwide and the search for adequate drugs remains a challenge and an important area of interest in the drug discovery field. This book addresses approaches to the treatment of retinal diseases, targeting common

processes and components. Despite their causative origins, which comprise genetic dystrophies, age-related degenerations, as well as pathologies associated with other diseases, a neurodegenerative component appears, sooner or later, in the course of the disease. As is the case for most neurodegenerative diseases, the available treatments are far from satisfactory. The aim of this book is to highlight research and drug development efforts in targeting such common processes as a potential path to provide treatments to the millions of affected people.

This book will contain the proceedings of the XIV International Symposium on Retinal Degeneration (RD2010), held July 13-17, 2010, in Mont-Tremblant, Quebec, Canada. The volume will present representative state-of-the-art research in almost all areas of retinal degenerations, ranging from cytopathologic, physiologic, diagnostic and clinical aspects; animal models; mechanisms of cell death; candidate genes, cloning, mapping and other aspects of molecular genetics; and developing potential therapeutic measures such as gene therapy and neuroprotective agents for potential pharmaceutical therapy.

1 Kevin Moses It is now 25 years since the study of the development of the compound eye in Drosophila really began with a classic paper (Ready et al. 1976). In 1864, August Weismann published a monograph on the development of Diptera and included some beautiful drawings of the developing imaginal discs (Weismann 1864). One of these is the first description of the third instar eye disc in which Weismann drew a vertical line

separating a posterior domain that included a regular pattern of clustered cells from an anterior domain without such a pattern. Weismann suggested that these clusters were the precursors of the adult ommatidia and that the line marks the anterior edge of the eye. In his first suggestion he was absolutely correct - in his second he was wrong. The vertical line shown was not the anterior edge of the eye, but the anterior edge of a moving wave of patterning and cell type specification that 112 years later (1976) Ready, Hansen and Benzer would name the "morphogenetic furrow". While it is too late to hear from August Weismann, it is a particular pleasure to be able to include a chapter in this Volume from the first author of that 1976 paper: Don Ready! These past 25 years have seen an astonishing explosion in the study of the fly eye (see Fig.

Central nervous system (CNS) development and post-injury neurogenesis require accurate coordination of neural stem cell proliferation, progenitor cell differentiation, neuron, glia migration and maturation, and synapse formation between axons and dendrites. Such systems with high complexity require strict temporal and spatial control via several levels of regulation, in which the transcription regulation is one of the most critical steps. The developmental and injury-repair process involves over 18,000 genes, for majority of which the molecular mechanism governing their transcription remains largely unknown. In an attempt to address this question, four projects were conducted focusing on two levels of transcription regulation: i.e., chromatin modification, and the interaction of cis-acting regulatory sequences with trans-acting protein factors.

Computational methods were adopted to analyze the sequences of the cis-elements and iii make predictions for their interacting transcription factors (TFs). The functional roles of these cis- and trans-elements were further determined in vivo and in vitro. The following findings are presented: 1) the function of DNA topoisomerase II beta (Top2b) in proper laminar formation and cell survival during retinal development; 2) the development of computational method for identifying gene regulatory networks involving enhancers and master TFs that are important in retinal cell differentiation; 3) the mechanism of Notch1 regulation in neural stem/progenitor cells via the interaction between Nkx6.1 and a CNS specific enhancer CR2 during the development of the spinal cord interneurons; and 4) the role of CR2 in aNSC activation after injury. Findings from this dissertation provide new insights into the molecular mechanisms underlying transcription regulation during CNS development and post-injury neurogenesis. They can also serve as a basis for future development of gene therapies and regenerative medicine for neurological disorders including spinal cord injury.
Recent advances in stem cell biology, nanotechnology and gene therapy have opened new avenues for therapeutics. The availability of molecular therapeutics that rely on the delivery of DNA, RNA or proteins, harnessing enhanced delivery with nanoparticles, and the regenerative potential of stem cells (adult, embryonic or induced pluripotent stem cells) has had a tremendous impact on translational medicine. The chapters in this book cover a range of strategies for molecular and cellular therapies for human

disease, their advantages, and central challenges to their widespread application. Potential solutions to these issues are also discussed in detail. Further, the book addresses numerous advances in the field of molecular therapeutics that will be of interest to the general scientific community. Lastly, the book provides specific examples of disease conditions for which these strategies have been transferred to the clinic. As such, it will be extremely useful for all students, researchers and clinicians working in the field of translational medicine and molecular therapeutics.

Pediatric retinal diseases are not simply retinal diseases that occur in children; rather, they are unique disorders that often are not found in adults. This textbook of the pediatric retina offers in-depth guidance on congenital and acquired diseases of the retina in the pediatric population. It is organized according to disease onset and timing, as well as anatomy. All chapters are written by leading authorities in the field from both the pediatric and the retinal perspective. A multidisciplinary approach to the topic is adopted, and critical information is included on disease classification and diagnosis, pathophysiology, genetics, complications, and prognosis. Pediatric Retina will be a useful source of information for pediatric ophthalmologists, retina specialists, and other eye care providers who care for children.