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Matthew M. LaVail • John D. Ash
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Christian Grimm
Editors

Retinal Degenerative Diseases

 Springer

Editors

Matthew M. LaVail
Anatomy and Ophthalmology
University of California
San Francisco School of Medicine
San Francisco, CA, USA
matthew.lavail@ucsf.edu

John D. Ash
Ophthalmology
College of Medicine
University of Oklahoma
Health Sciences Center
Oklahoma City, OK, USA
John-Ash@ouhsc.edu

Robert E. Anderson
Ophthalmology and Cell Biology
Dean A. McGee Eye Institute
University of Oklahoma
Health Sciences Center
Oklahoma City, OK, USA
Robert-Anderson@ouhsc.edu

Joe G. Hollyfield
Ophthalmology
Cole Eye Institute at the Cleveland Clinic
Cleveland, OH, USA
hollyfj@ccf.org

Christian Grimm
Experimental Ophthalmology
University of Zurich
Zurich, Switzerland
cgrimm@opht.uzh.ch

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Michael Danciger, Ph.D.
1943–2010

Michael Danciger was a pioneer in the identification of modifier genes of complex genetic disorders that lead to blindness. The impact of his research and collaborations continues to be felt and expanded. Equally important was his delightful personal quality of leaving an indelible mark on everyone he met and with whom he worked. Michael was a long-time supporter of the RD Symposia. He is missed, and we are honored to dedicate this proceedings volume to him.



Robert B. Barlow, Jr., Ph.D.
1939–2009

Robert “Bob” Barlow was an extremely energetic man who contributed to many areas of research in vision and neurobiological science and held many responsible positions that influenced numerous investigators. He was a long-time supporter of the RD Symposia, as was his wife, Pat, pictured here together at the RD2004 meeting in Australia. He is missed, and we are honored to dedicate this proceedings volume to him.

Preface

The International Symposia on Retinal Degeneration have been held in conjunction with the biennial International Congress of Eye Research (ICER) since 1984. These RD Symposia have allowed basic and clinician scientists from around the world to convene and present their new research findings. They have been organized to allow sufficient time for discussions and one-on-one interactions in a relaxed atmosphere, where international friendships and collaborations can be fostered.

The XIV International Symposium on Retinal Degeneration (also known as RD2010) was held from July 13–17, 2010 at the Fairmont Tremblant Hotel in the resort village of Mont-Tremblant, Quebec, Canada. The meeting brought together 232 basic and clinician scientists, retinal specialists in ophthalmology, and trainees in the field from all parts of the world. In the course of the meeting, 38 platform and 134 poster presentations were given, and a majority of these are presented in this proceedings volume. New discoveries and state-of-the-art findings from most research areas in the field of retinal degenerations were presented. This was the largest of all of the RD Symposia, with the greatest number of attendees and presentations.

The RD2010 meeting was highlighted by three special plenary lectures. The first was given by *Elise Héon*, MD, of the University of Toronto Hospital for Sick Children, Toronto, Ontario, Canada. Dr. Heon discussed “What Bardet-Biedl Syndrome teaches us about ciliopathies.” The second was given by *Gregory Hageman*, PhD, of the John Moran Eye Center, University of Utah, Salt Lake City, UT. Dr. Hageman described a “New era in the understanding of age-related macular degeneration.” The third plenary lecture was given by *Jayakrishna Ambati*, MD, of the University of Kentucky, Lexington, KY. Dr. Ambati discussed “Age-related macular degeneration and the other double helix.”

We thank the outstanding management and staff of the beautiful Fairmont Tremblant Hotel for all of their assistance in making this an exceptionally smooth-running conference and a truly memorable experience for all of the attendees. These included, in particular, *Isabelle Gilbert*, *Émilie Normandeau*, and *Patrick Skelly*. We also thank *Jonathan Marier* of AVW-TELAV for providing audio/visual equipment and services that resulted in a flawless flow of platform presentations. Lastly, we thank *Steven LeFort* and, particularly, *Marie-Chantal Thibault* of JPdL Tremblant

for their planning and implementing transportation of most of the attendees to and from Montreal and the meeting venue in Mont-Tremblant, as well as all aspects of the end-of-meeting Gala at the beautiful “Summit” overlooking Mont-Tremblant and the majestic Laurentian mountains, with a truly Canadian meal, music, and festivities.

The Symposium received international financial support from a number of organizations. We are particularly pleased to thank The Foundation Fighting Blindness, Columbia, Maryland, for its continuing support of this and all previous biennial Symposia, without which we could not have held these important meetings. In addition, for the fifth time, the National Eye Institute of the National Institutes of Health contributed in a major way to the meeting. In the past, funds from these two organizations allowed us to provide 25–35 Travel Awards to young investigators and trainees working in the field of retinal degenerations. However, the response to the Travel Awards program was extraordinary, with 94 applicants, many more than in the past. For this reason, we sought additional support for the Travel Awards program. The Foundation Fighting Blindness-Canada/Institute of Genetics was a major contributor, and for the first time, we turned to industry sponsors and received generous contributions from Novartis Pharma AG, Alcon, Genentech, Inspire Pharmaceuticals, Pfizer, Inc., Genzyme and Bioptigen, Inc. In total, we were able to fund 42 Travel Awards, the largest number ever for these Symposia. Many of the contributing foundations and industry sponsors sent one to several members of their organization to attend the meeting. Their participation and comments in the scientific sessions were instructive to many, offering new perspectives to some of the problems being discussed.

There were two additional “firsts” for the RD Symposia at RD2010. For the first time, there was a commercial exhibitor at the Symposium, Bioptigen, which demonstrated its Spectral Domain Ophthalmic Imaging System (OCT) for small laboratory animals; this was highly instructive for many of the attendees. Second, the world-famous Tremblant International Blues Festival (17th Edition) was held during the RD2010 meeting, immediately adjacent to the Fairmont Tremblant venue. With almost continuous free performances every evening of the Symposium, many groups of attendees enjoyed these together after dinner.

We also acknowledge the diligent and outstanding efforts of Ms. *Holly Whiteside*, who carried out most of the administrative aspects of the RD2010 Symposium, designed and maintained the meeting website, and participated in the production of this volume. Holly is the Administrative Manager of Dr. Anderson’s laboratory at the University of Oklahoma Health Sciences Center, and she has become the permanent Coordinator for the Retinal Degeneration Symposia. Her dedicated efforts with the Symposia since RD2000 have provided continuity not available previously, and we are deeply indebted to her. Also, Dr. *Michael Matthes* in Dr. LaVail’s laboratory played a major role in all aspects in the production of this volume, along with the assistance of Ms. *Kelly Ahern*, in Dr. LaVail’s laboratory.

Recognizing the need to bring younger individuals into the organizational structure of the RD Symposia, at the RD2008 meeting, we invited Drs. *John Ash* and *Christian Grimm* to become members of the organizing committee. Thus, instead of

the rotating head organizer working mostly with Holly Whiteside to organize the meeting and prepare the proceedings volume, Dr. Ash assumed equal responsibility with Dr. LaVail for both efforts for RD2010. Dr. *Anderson* continued in his role as financial administrator for each of the Symposia, working through the Dean McGee Eye Institute, which generously provides the financial responsibility for the meetings and the mechanism for registration of participants. We were pleased to announce at the Gala at RD2010 that our third new member of the organizing committee is Dr. *Cathy Bowes Rickman*. Dr. Grimm will work closely with Dr. Hollyfield for the RD2012 meeting to be held in Germany.

Finally, we honor the memory of two colleagues who died during the preparation of the RD2010 meeting in 2009 and 2010. *Michael Danciger* was a great friend to most who attend our RD meetings. *Robert Barlow*, likewise, was a long-time attendee of the RD Symposia. Both were outstanding scientists and are missed. We dedicate this volume to Michael and Bob.

San Francisco, CA, USA
Oklahoma City, OK, USA
Oklahoma City, OK, USA
Cleveland, OH, USA
Zurich, Switzerland

Matthew M. LaVail
John D. Ash
Robert E. Anderson
Joe G. Hollyfield
Christian Grimm

Contents

Part I AMD: Basic Mechanisms, Inflammation and Immunity

1	A Window to Innate Neuroimmunity: Toll-Like Receptor-Mediated Cell Responses in the Retina	3
	Mark E. Kleinman and Jayakrishna Ambati	
2	Autoimmune Biomarkers in Age-Related Macular Degeneration: A Possible Role Player in Disease Development and Progression	11
	Alessandro Iannaccone, Indira Neeli, Pratheebha Krishnamurthy, Nataliya I. Lenchik, Haibao Wan, Ivan C. Gerling, Dominic M. Desiderio, and Marko Z. Radic	
3	Local Vs. Systemic Mononuclear Phagocytes in Age-Related Macular Degeneration and Their Regulation by <i>CCL2-CCR2</i> and <i>CX3CL1-CX3CR1</i> Chemokine Signalling	17
	Ulrich F.O. Luhmann and Robin R. Ali	
4	Sublytic Membrane-Attack-Complex Activation and VEGF Secretion in Retinal Pigment Epithelial Cells	23
	Kannan Kunchithapautham, Mausumi Bandyopadhyay, Mohammad Dahrouj, Joshua M. Thurman, and Bärbel Rohrer	
5	Complement Activation in Retinal Degeneration	31
	Matt Rutar, Riccardo Natoli, Jan Provis, and Krisztina Valter	
6	Microglia in the Outer Retina and Their Relevance to Pathogenesis of Age-Related Macular Degeneration	37
	Wenxin Ma, Lian Zhao, and Wai T. Wong	

7	Lutein or Zeaxanthin Supplementation Suppresses Inflammatory Responses in Retinal Pigment Epithelial Cells and Macrophages	43
	Qingning Bian, Tingyu Qin, Zhihong Ren, Dayong Wu, and Fu Shang	
8	Exploring the Potential Role of the Oxidant-Activated Transcription Factor Aryl Hydrocarbon Receptor in the Pathogenesis of AMD	51
	Goldis Malek, Mary Dwyer, and Donald McDonnell	
9	Common Mechanisms for Separate Maculopathies?	61
	Elod Kortvely and Marius Ueffing	
10	The Role of Amyloid-β in Retinal Degeneration	67
	Julien Bruban, Virginie Dinet, and Frédéric Mascarelli	
11	Molecule-Specific Imaging and Quantitation of A2E in the RPE	75
	Zsolt Ablonczy, Danielle B. Gutierrez, Angus C. Grey, Kevin L. Schey, and Rosalie K. Crouch	
12	Autophagy in the Retina: A Potential Role in Age-Related Macular Degeneration	83
	Sayak K. Mitter, Haripriya Vittal Rao, Xiaoping Qi, Jun Cai, Andrew Sugrue, William A. Dunn Jr., Maria B. Grant, and Michael E. Boulton	
Part II Neuroprotection, Drugs and Novel Protective Therapies		
13	Regeneration of Cone Outer Segments Induced by CNTF	93
	Rong Wen, Weng Tao, Lingyu Luo, Deqiang Huang, Konrad Kauper, Paul Stabila, Matthew M. LaVail, Alan M. Laties, and Yiwen Li	
14	Glucocorticoid-Dependent Mechanisms in Photoreceptor Survival	101
	Marisa A. Cubilla, Mauricio M. Castañeda, Tomás P. Bachor, and Angela M. Suburo	
15	HDAC Inhibition Prevents <i>Rd1</i> Mouse Photoreceptor Degeneration	107
	Javier Sancho-Pelluz and François Paquet-Durand	
16	Neuroprotective Dose Response in RCS Rats Implanted with Microphotodiode Arrays	115
	Machelle T. Pardue, Moon K. Kim, Tiffany A. Walker, Amanda E. Faulkner, Alan Y. Chow, and Vincent T. Ciavatta	

17	Treatment with 670-nm Light Protects the Cone Photoreceptors from White Light-Induced Degeneration	121
	Rizalyn S. Albarracin and Krisztina Valter	
18	Dark-Rearing the <i>rd10</i> Mouse: Implications for Therapy	129
	Therese Cronin, Arkady Lyubarsky, and Jean Bennett	
19	Intravitreal Injection of Erythropoietin Glycosylation Analogs Does Not Protect Rod Photoreceptor Cells from Light-Induced Damage	137
	Masaki Tanito, Feng Li, and Robert E. Anderson	
20	Relieving Bottlenecks in RNA Drug Discovery for Retinal Diseases	145
	Jack M. Sullivan, Edwin H. Yau, R. Thomas Taggart, Mark C. Butler, and Tiffany A. Kolniak	
21	On Further Development of Barrier Modulation as a Technique for Systemic Ocular Drug Delivery	155
	Finnian Hanrahan, Matthew Campbell, Anh T. Nguyen, Mayu Suzuki, Anna-Sophia Kiang, Lawrence C. Tam, Oliviero L. Gobbo, Sorcha Ní Dhubhghaill, Marian M. Humphries, Paul F. Kenna, and Pete Humphries	
22	An Application for Mammalian Optic Nerve Repair by Fish Regeneration-Associated Genes	161
	Yoshiki Koriyama, Kayo Sugitani, Toru Matsukawa, and Satoru Kato	
23	The Mechanism of Fenretinide (4-HPR) Inhibition of β-carotene Monooxygenase 1. New Suspect for the Visual Side Effects of Fenretinide	167
	Eugenia Poliakov, Alexander Gubin, James Laird, Susan Gentleman, Robert G. Salomon, and T. Michael Redmond	
Part III Gene Therapy		
24	Gene Augmentation Trials Using the Rpe65-Deficient Dog: Contributions Towards Development and Refinement of Human Clinical Trials	177
	Simon M. Petersen-Jones, Matthew J. Annear, Joshua T. Bartoe, Freya M. Mowat, Susie E. Barker, Alexander J. Smith, James W. Bainbridge, and Robin R. Ali	
25	Gene Therapy Restores Missing Cone-Mediated Vision in the CNGA3^{-/-} Mouse Model of Achromatopsia	183
	Stylianos Michalakis, Regine Mühlfriedel, Naoyuki Tanimoto, Vidhyasankar Krishnamoorthy, Susanne Koch, M. Dominik Fischer, Elvir Becirovic, Lin Bai, Gesine Huber, Susanne C. Beck, Edda Fahl, Hildegard Büning, Jennifer Schmidt, Xiangang Zong, Tim Gollisch, Martin Biel, and Mathias W. Seeliger	

26 Functional Rescue of P23H Rhodopsin Photoreceptors by Gene Delivery 191
 Marina S. Gorbatyuk, Oleg S. Gorbatyuk, Matthew M. LaVail, Jonathan H. Lin, William W. Hauswirth, and Alfred S. Lewin

27 Gene Delivery of Wild-Type Rhodopsin Rescues Retinal Function in an Autosomal Dominant Retinitis Pigmentosa Mouse Model 199
 Haoyu Mao, Marina S. Gorbatyuk, William W. Hauswirth, and Alfred S. Lewin

28 Retinal Degeneration and Cellular Suicide 207
 Wai Gin Fong and Catherine Tsilfidis

29 Suppression of *rd5* Expression by siRNA and Gene Replacement Strategies for Gene Therapy Using rAAV Vector 215
 Hilda Petrs-Silva, Douglas Yasumura, Michael T. Matthes, Matthew M. LaVail, Alfred S. Lewin, and William W. Hauswirth

30 Silencing the Expression of *CTRP5/C1QTNF5* and *ELOVL4* Genes by Small Interfering RNA..... 225
 Venkata Ramana Murthy Chavali, Vidyullatha Vasireddy, and Radha Ayyagari

31 Gene Therapy Strategies for Usher Syndrome Type 1B..... 235
 David S. Williams and Vanda S. Lopes

Part IV Blood Vessels, Angiogenesis, and Neovascularization

32 Neovascularization: Ocular Diseases, Animal Models and Therapies 245
 Xue Cai, Steven A. Sezate, and James F. McGinnis

33 Retinal Neovascular Disorders: Mouse Models for Drug Development Studies..... 253
 Rosanne M. Yetemian and Cheryl M. Craft

34 A Review and Update on the Molecular Basis of Pathogenesis of Sorsby Fundus Dystrophy..... 261
 Heidi Stöhr and Bela Anand-Apte

35 The Importance of Hypoxia-Regulated, RPE-Targeted Gene Therapy for Choroidal Neovascularization 269
 George W. Smith, C. Kathleen Dorey, Howard Prentice, and Janet Blanks

36 What Is the Role of CCR3 in Choroidal Neovascularization?..... 279
 Yiwen Li, Deqiang Huang, Xin Xia, Zhengying Wang, Lingyu Luo, and Rong Wen

37 Intermittent But Not Constant High Glucose Induces ER Stress and Inflammation in Human Retinal Pericytes..... 285
 Yimin Zhong, Joshua J. Wang, and Sarah X. Zhang

38 Regulation of Retinal Vascular Permeability by Betacellulin..... 293
 Masahiko Sugimoto, Alecia Cutler, Gregory Grossman, and Bela Anand-Apte

39 Presence of RPE-Produced VEGF in a Timely Manner Is Critical to Choroidal Vascular Development 299
 Meili Zhu, Yanyan Bai, Lixin Zheng, and Yun-Zheng Le

40 Vasohibin-1 and Retinal Pigment Epithelium 305
 Yumi Ishikawa, Nobuhiro Nagai, Hideyuki Onami, Norihiro Kumasaka, Ryosuke Wakusawa, Hikaru Sonoda, Yasufumi Sato, and Toshiaki Abe

Part V Genotype/Phenotype

41 Polymorphic Variation of RPGRIP1L and IQCB1 as Modifiers of X-Linked Retinitis Pigmentosa Caused by Mutations in RPGR..... 313
 Abigail T. Fahim, Sara J. Bowne, Lori S. Sullivan, Kaylie D. Webb, Jessica T. Williams, Dianna K. Wheaton, David G. Birch, and Stephen P. Daiger

42 RPGRIP1 and Cone-Rod Dystrophy in Dogs..... 321
 Tatyana Kuznetsova, Barbara Zangerl, and Gustavo D. Aguirre

43 High-Throughput Approaches for the Genetic Diagnosis of Retinal Dystrophies 329
 Esther Pomares, Gemma Marfany, and Roser González-Duarte

44 Genes and Mutations in Autosomal Dominant Cone and Cone-Rod Dystrophy 337
 Susanne Kohl, Veronique Kitiratschky, Monika Papke, Simone Schaich, Alexandra Sauer, and Bernd Wissinger

45 The Power of Homozygosity Mapping: Discovery of New Genetic Defects in Patients with Retinal Dystrophy 345
 Karin W. Littink, Anneke I. den Hollander, Frans P.M. Cremers, and Rob W.J. Collin

46 Development and Validation of a Canine-Specific Profiling Array to Examine Expression of Pro-apoptotic and Pro-survival Genes in Retinal Degenerative Diseases..... 353
 Sem Genini, William A. Beltran, and Gustavo D. Aguirre

47 The Chromosome 10q26 Susceptibility Locus in Age-Related Macular Degeneration 365
 Chloe M. Stanton, Kevin J. Chalmers, and Alan F. Wright

48 Congenital Stationary Night Blindness: Mutation Update and Clinical Variability..... 371
 Nidhi Lodha, Catrina M. Loucks, Chandree Beaulieu, Jillian S. Parboosingh, and N. Torben Bech-Hansen

49 Serum Biomarkers and Trafficking Defects in Peripheral Tissues Reflect the Severity of Retinopathy in Three Brothers Affected by Choroideremia 381
 Natalia Strunnikova, Wadih M. Zein, Chris Silvin, and Ian M. MacDonald

Part VI New Animal Models of Retinal Degeneration

50 Translational Vision Research Models Program..... 391
 Jungyeon Won, Lan Ying Shi, Wanda Hicks, Jieping Wang, Juergen K. Naggert, and Patsy M. Nishina

51 Zebrafish: A Model System for the Investigation of Novel Treatments for Retinal Disease 399
 Cheryl Y. Gregory-Evans

52 Retinal Degeneration in the Fly 407
 Nansi Jo Colley

53 Looking into Eyes: Rhodopsin Pathologies in *Drosophila*..... 415
 Ana Griciuc, Liviu Aron, and Marius Ueffing

54 Müller Glia as a Source of Neuronal Progenitor Cells to Regenerate the Damaged Zebrafish Retina..... 425
 Craig M. Nelson and David R. Hyde

55 The Genetics of Outer Segment Morphogenesis in Zebrafish 431
 Alison L. Reynolds, Oliver E. Blacque, and Breandán N. Kennedy

56 Factor XIII^A Induction in the Retina and Optic Nerve After Optic Nerve Lesion in Goldfish..... 443
 Kayo Sugitani, Kazuhiro Oogai, Hiroshi Nakashima, and Satoru Kato

Part VII Analysis of Retinal Degeneration by Imaging and Functional Testing

57 Imaging the Photoreceptor Mosaic with Adaptive Optics: Beyond Counting Cones 451
 Pooja Godara, Melissa Wagner-Schuman, Jungtae Rha, Thomas B. Connor Jr., Kimberly E. Stepien, and Joseph Carroll

58 Baseline Imaging Reveals Preexisting Retinal Abnormalities in Mice 459
 Brent A. Bell, Charles Kaul, Mary E. Rayborn, and Joe G. Hollyfield

59 Correlation Between Spectral Domain OCT Retinal Nerve Fibre Layer Thickness and Multifocal Pattern Electroretinogram in Advanced Retinitis Pigmentosa 471
 Ieva Sliesoraityte, Eric Troeger, Antje Bernd, Anne Kurtenbach, and Eberhart Zrenner

60 Imaging Human Postmortem Eyes with SLO and OCT 479
 Nika Bagheri, Brent A. Bell, Vera L. Bonilha, and Joe G. Hollyfield

61 In Vivo Assessment of Rodent Retinal Structure Using Spectral Domain Optical Coherence Tomography 489
 M. Dominik Fischer, Gesine Huber, Francois Paquet-Durand, Peter Humphries, T. Michael Redmond, Christian Grimm, and Mathias W. Seeliger

62 Rod Photoreceptor Temporal Properties in Retinal Degenerative Diseases 495
 Yuquan Wen, Kirsten G. Locke, Donald C. Hood, and David G. Birch

63 ERG Critical Flicker Frequency Assessment in Humans 503
 Kristen E. Bowles and Timothy W. Kraft

Part VIII Mechanisms of Retinal Degeneration

64 Biology of Retinoschisin 513
 Camasamudram Vijayasarathy, Lucia Ziccardi, and Paul A. Sieving

65 Transcriptome Analyses to Investigate the Pathogenesis of RNA Splicing Factor Retinitis Pigmentosa 519
 Michael H. Farkas, Greg R. Grant, and Eric A. Pierce

66 The Role of the X-linked Retinitis Pigmentosa Protein RP2 in Vesicle Traffic and Cilia Function 527
 Nele Schwarz, Alison J. Hardcastle, and Michael E. Cheetham

67 *Caenorhabditis elegans* as a Model Organism for Ciliopathies and Related Forms of Photoreceptor Degeneration 533
 Calvin A. Mok and Elise Héon

68 Towards a Pathological Mechanism for IMPDH1-Linked Retinitis Pigmentosa 539
 Dharia A. McGrew and Lizbeth Hedstrom

69 Calpain and Photoreceptor Apoptosis..... 547
 Anh T.H. Nguyen, Matthew Campbell, Paul F. Kenna,
 Anna-Sophia Kiang, Lawrence Tam, Marian M. Humphries,
 and Peter Humphries

70 Ceramide Signaling in Retinal Degeneration..... 553
 Hui Chen, Julie-Thu A. Tran, Richard S. Brush, Anisse Saadi,
 Abul K. Rahman, Man Yu, Douglas Yasumura, Michael T. Matthes,
 Kelly Ahern, Haidong Yang, Matthew M. LaVail,
 and Md Nawajes A. Mandal

**71 Endoplasmic Reticulum-Associated Degradation (ERAD)
 of Misfolded Glycoproteins and Mutant P23H Rhodopsin
 in Photoreceptor Cells** 559
 Heike Kroeger, Wei-Chieh Chiang, and Jonathan H. Lin

**72 Protein Misfolding and Potential Therapeutic Treatments
 in Inherited Retinopathies**..... 567
 Lawrence C.S. Tam, Anna-Sophia Kiang, Matthew Campbell,
 James Keane, G. Jane Farrar, Marian M. Humphries,
 Paul F. Kenna, and Pete Humphries

**73 Development of a Cellular Model of Rod Opsin
 Retinitis Pigmentosa** 573
 Matthew Adamowicz, Antonius Song, Samuel Wadsworth,
 Abraham Scaria, and Catherine O’Riordan

**74 A Brief Account of Rho GTPases in Retinal Physiology
 and Pathophysiology**..... 581
 Severin Reinhard Heynen, Omolara O. Ogunshola,
 and Christian Grimm

75 Molecular Clues to Bothnia-Type Retinal Dystrophy..... 589
 Xiaoqin He, Joel Lobsiger, and Achim Stocker

**76 A Novel Missense Mutation in Both *OPNILW* and *OPNIMW*
 Cone Opsin Genes Causes X-Linked Cone
 Dystrophy (*XL COD5*)**..... 595
 Jessica C. Gardner, Tom R. Webb, Naheed Kanuga,
 Anthony G. Robson, Graham E. Holder, Andrew Stockman,
 Caterina Ripamonti, Neil D. Ebenezer, Olufunmilola Ogun,
 Sophie Devery, Genevieve A. Wright, Eamonn R. Maher,
 Michael E. Cheetham, Anthony T. Moore, Michel Michaelides,
 and Alison J. Hardcastle

77 A Potential Cytosolic Function of Bestrophin-1 603
 Olaf Strauß, Rudgar Neussert, Claudia Müller,
 and Vladimir M. Milenkovic

78 Modeling the Structural Consequences of *BEST1* Missense Mutations 611
 Karina E. Guziewicz, Gustavo D. Aguirre, and Barbara Zanger

79 Microglial Activation and Transcriptomic Changes in the Blue Light-Exposed Mouse Retina 619
 Stefanie Ebert, Yana Walczak, Charlotte Remé, and Thomas Langmann

80 Overexpression of ROM-1 in the Cone-Dominant Retina 633
 Dibyendu Chakraborty, Shannon M. Conley, Zack Nash, Xi-Qin Ding, and Muna I. Naash

81 Analysis of the RPE Sheet in the rd10 Retinal Degeneration Model 641
 Micah A. Chrenek, Nupur Dalal, Christopher Gardner, Hans Grossniklaus, Yi Jiang, Jeffrey H. Boatright, and John M. Nickerson

82 Networks Modulating the Retinal Response to Injury: Insights from Microarrays, Expression Genetics, and Bioinformatics 649
 Félix R. Vázquez-Chona and Eldon E. Geisert

83 Mislocalization of Oligomerization-Incompetent RDS is Associated with Mislocalization of Cone Opsins and Cone Transducin 657
 Shannon M. Conley, Dibyendu Chakraborty, and Muna I. Naash

84 HSP70 Gene Expression in the Zebrafish Retina After Optic Nerve Injury: A Comparative Study Under Heat Shock Stresses 663
 Chieko Fujikawa, Mikiko Nagashima, Kazuhiro Mawatari, and Satoru Kato

Part IX Retinal Development, Physiology, Cell and Molecular Biology

85 Restoration of Retinal Development in *Vsx2* Deficient Mice by Reduction of *Gdf11* Levels 671
 Rosaysela Santos, Jeffry Wu, Jason A. Hamilton, Rita Pinter, Robert Hindges, and Anne L. Calof

86 The Different Functions of Norrin 679
 Barbara M. Braunger and Ernst R. Tamm

87 Roles of Homeobox Genes in Retinal Ganglion Cell Differentiation and Axonal Guidance 685
 Qi Zhang and David D. Eisenstat

88	Unraveling the Molecular Mystery of Retinal Pigment Epithelium Phagocytosis	693
	Nora B. Caberoy and Wei Li	
89	Isolating Photoreceptor Compartment-Specific Protein Complexes for Subsequent Proteomic Analysis	701
	Gregory H. Grossman, Gayle J.T. Pauer, George Hoppe, and Stephanie A. Hagstrom	
90	Expression of the Integrin Coreceptor Transglutaminase-2 in the RPE In Vivo and in Culture	709
	Linda Ruggiero and Silvia C. Finnemann	
91	On Your Marks... Get Bound... Internalize!	717
	Ah-Lai Law and Emeline F. Nandrot	
92	Endo-Lysosome Function in the Retinal Pigment Epithelium in Health and Disease	723
	Aparna Lakkaraju	
93	$\alpha\beta 5$ Integrin-Dependent Diurnal Phagocytosis of Shed Photoreceptor Outer Segments by RPE Cells Is Independent of the Integrin Coreceptor Transglutaminase-2	731
	Linda Ruggiero, Zsolt Sarang, Zsuzsa Szondy, and Silvia C. Finnemann	
94	Trafficking of Presynaptic PMCA Signaling Complexes in Mouse Photoreceptors Requires Cav1.4 α_1 Subunits	739
	Wei Xing, Abram Akopian, and David Križaj	
95	Roles for AMP-Activated Protein Kinase in RPE Cell Function	745
	Suofu Qin	
96	Genome-Wide Occupancy Analysis by ChIP-chip and ChIP-Seq	753
	Hong Hao	
97	The Bisretinoids of RPE Lipofuscin: A Complex Mixture	761
	Janet R. Sparrow and Kazunori Yamamoto	
98	Biochemical Characterization of Cone Cyclic Nucleotide-Gated (CNG) Channel Using the Infrared Fluorescence Detection System	769
	Xi-Qin Ding, Alexander Matveev, Anil Singh, Naoka Komori, and Hiroyuki Matsumoto	
99	Ras-Associating Domain Proteins: A New Class of Cyclic Nucleotide-Gated Channel Modulators	777
	Vivek K. Gupta, Ammaji Rajala, and Raju V.S. Rajala	

Dharia McGrew
Calvin Mok
Anh Thi Hong Nguyen
Francisco Javier Sancho-Pelluz
Rosaysela Santos
Simone Schimpf-Linzenbold
Hilda Petrs Silva
George Wesley Smith
Chloe Stanton
Masahiko Sugimoto
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Part I
**AMD: Basic Mechanisms, Inflammation
and Immunity**

Chapter 5

Complement Activation in Retinal Degeneration

Matt Rutar, Riccardo Natoli, Jan Provis, and Krisztina Valter

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5.1 Introduction

The complement system is a component of the innate arm of the immune response which provides a rapid host defence against a range of immunological challenges. Through a cascade of enzymatic cleavages culminating chiefly in the deposition of complement component 3 (C3) on activating surfaces, the activity of complement enhances the ability of the host to initiate defence against infection, and clear immune complexes, apoptotic cells and other noxious substances (Gasque 2004).

M. Rutar (✉) • K. Valter

Departments of Research School of Biology, ARC Centre of Excellence in Vision Science, The Australian National University, RN Robertson Bldg, Sullivan's Creek Rd, Canberra, ACT 0200, Australia

ARC Centre of Excellence in Vision Science, The Australian National University, Canberra, ACT 0200, Australia
e-mail: matt.rutar@rsbs.anu.edu.au

R. Natoli

ANU Medical School, The Australian National University, Canberra, ACT 0200, Australia

J. Provis

Departments of Research School of Biology, ARC Centre of Excellence in Vision Science, The Australian National University, RN Robertson Bldg, Sullivan's Creek Rd, Canberra, ACT 0200, Australia

ARC Centre of Excellence in Vision Science, The Australian National University, Canberra, ACT 0200, Australia

ANU Medical School, The Australian National University, Canberra, ACT 0200, Australia

If activated in an exaggerated and inappropriate manner, however, complement also has the capacity to destroy host tissue. Indeed, pathogenic complement activation is directly implicated in neuronal damage following intracerebral haemorrhage (Yang et al. 2006), and post-ischemic cerebral injury (Figueroa et al. 2005). Recently, complement activation has been found to play a role in age-related macular degeneration (AMD), a retinal disease affecting central vision and a leading cause of blindness in the western world. Gene association studies have identified a number of polymorphisms in complement genes, including CFH (Klein et al. 2005), CFB (Jakobsdottir et al. 2008), C2 (Jakobsdottir et al. 2008) and C3 (Yates et al. 2007; Despret et al. 2009), which are strongly associated with the incidence of AMD.

We aimed to investigate whether a common role of complement in retinal dystrophy exists, by comparing the expression of the central component C3 in conjunction with cell death in a range of mechanistically distinct degenerative models. These include acute degeneration induced through either excessive light or hyperoxia, and chronic degeneration using the retinitis pigmentosa-mimicking P23H-3 rodent strain.

5.2 Methods

In the light damage model, young adult albino Sprague Dawley (SD) rats were exposed to a light intensity of 1,000 lx for a period of up to 24 h, after which some animals were kept in dim light (5 lx) to recover. At specific time points during (1, 3, 6, 12, 17 and 24 h) and after exposure (3, and 7 days), animals were euthanized and retinas extracted for analysis of C3 mRNA expression, and for counts of apoptotic cells in the outer nuclear layer (ONL) using the TUNEL technique. In the hyperoxia model, adult C57 mice were subjected to 75% oxygen for up to 14 days. Animals were euthanized after 3, 7 and 14 days, whereby retinas were dissected for the analysis of C3 mRNA expression, and TUNEL. In the P23H model, animals from line 3 of this transgenic strain were born and reared until postnatal day 50–130. Age-matched, non-degenerative SD rodents served as the control tissue. At this time, animals were euthanized and retinas extracted for analysis of C3 mRNA expression and TUNEL. C3 mRNA expression levels were determined by quantitative PCR (qPCR), where the assessment of the relative fold change was determined using the $\Delta\Delta C^q$, with GAPDH serving as the reference gene.

5.3 Results

In light-induced degeneration, qPCR results show that expression levels of complement C3 in the retina (Fig. 5.1) increased significantly over the course of 24 h bright light and continued to increase robustly into the post-exposure period to reach a peak differential expression of 1,038% compared to dim-reared animals. Photoreceptor death (TUNEL) dramatically increased by 24 h bright light exposure,

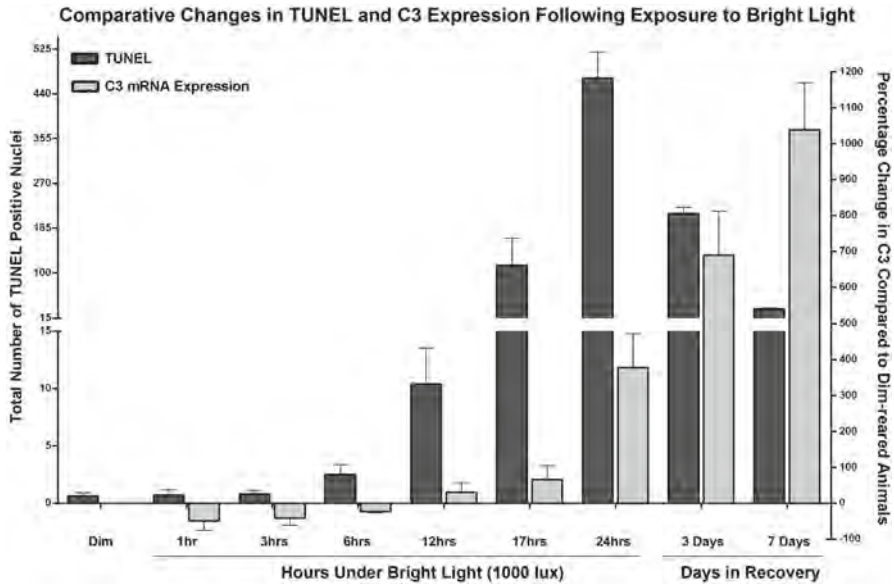


Fig. 5.1 C3 mRNA expression and TUNEL frequency during and following exposure to 1000 lx light. C3 expression during light damage showed a marked up-regulation by 24 h of exposure, which increased further in the ensuing post-exposure period. The number of TUNEL positive nuclei increased dramatically after 24 h of exposure, coinciding with the increase in C3 expression. In the post exposure period, cell death tapered off rapidly though persisted at a relatively low level after 7 days

consistent with the onset of C3 upregulation over the same period (Fig. 5.1). During exposure to hyperoxia, the expression of C3 did not increase after 3 and 7 days (Fig. 5.2). By 14 days, however, C3 expression increased to 180% compared to control animals. The number of TUNEL-positive photoreceptors increased moderately after 7 days hyperoxia, followed by a dramatic increase by 14 days (Fig. 5.2); coinciding with the upregulation of C3. In the retinas of P23H rats, an increase of 80% in C3 expression compared to SD rats was observed, while photoreceptor death was found to be higher in the P23H strain than the SD (Fig. 5.3).

5.4 Discussion

Our results indicate that while the degenerative stimuli in these models differ, an increased expression of C3 mRNA – which fuels the complement cascade – occurs in close association with substantial increases in photoreceptor cell death. Correspondingly, we provide evidence for a common pathway in retinal degeneration involving the activation of complement in response to the induction of photoreceptor injury and death. Our data are consistent with the implication of the

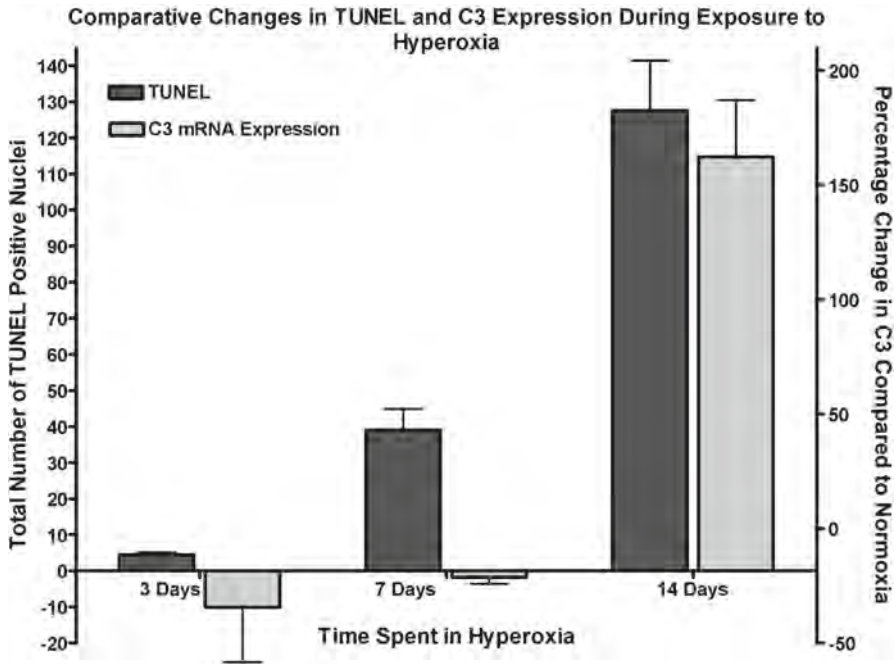


Fig. 5.2 C3 mRNA expression and TUNEL frequency in retinas during exposure to hyperoxia. No increase in C3 expression was evident during the early phases of hyperoxia, although this progressed to a substantial up-regulation by 14 days. A steady increase in TUNEL positive cells was apparent over the 14-day time course, reaching a peak at 14 days hyperoxia

complement system in the clearance of noxious substances, such as apoptotic cells (Gasque 2004; Trouw et al. 2008); which suggests that complement activation is geared toward a beneficial role in the maintenance of retinal homeostasis. This is at odds, however, with the association of complement with the incidence of AMD (Anderson et al. 2010). One explanation is that while complement may be activated to necessitate the efficient clearance of noxious substances, its exaggerated activation in the injured retina – aided by an abundance of apoptotic cells and other activating surfaces – induces further retinal degeneration through propagating an inappropriate inflammatory response (Walport 2001). Indeed, a recent investigation by Rohrer and colleagues has shown that complement activation exacerbates photoreceptor death in light-induced retinal degeneration (Rohrer et al. 2007). While direct evidence for complement-induced pathology in hyperoxia and the degenerative P23H strain are currently lacking, increased synthesis in C3 expression in both models following degeneration – consistent with light damage – supports the existence of a common detrimental role of complement activation in retinal degeneration. Consequently, anticomplement strategies may have a broad therapeutic potential in the treatment of various forms of retinal degeneration.

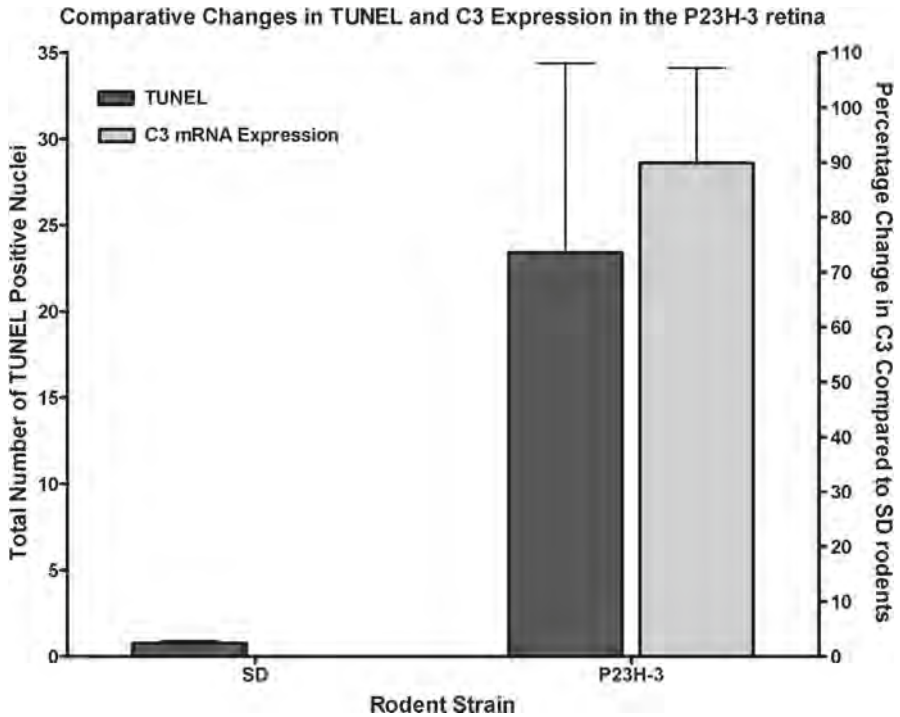


Fig. 5.3 C3 mRNA expression and TUNEL frequency in retinas from SD and degenerative P23H-3 rodents. C3 expression was elevated in retinas from P23H-3 animals compared to those of the SD strain. TUNEL analysis indicated an increase photoreceptor apoptosis in the P23H-3 retina compared to the SD – as expected for this degenerative strain

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