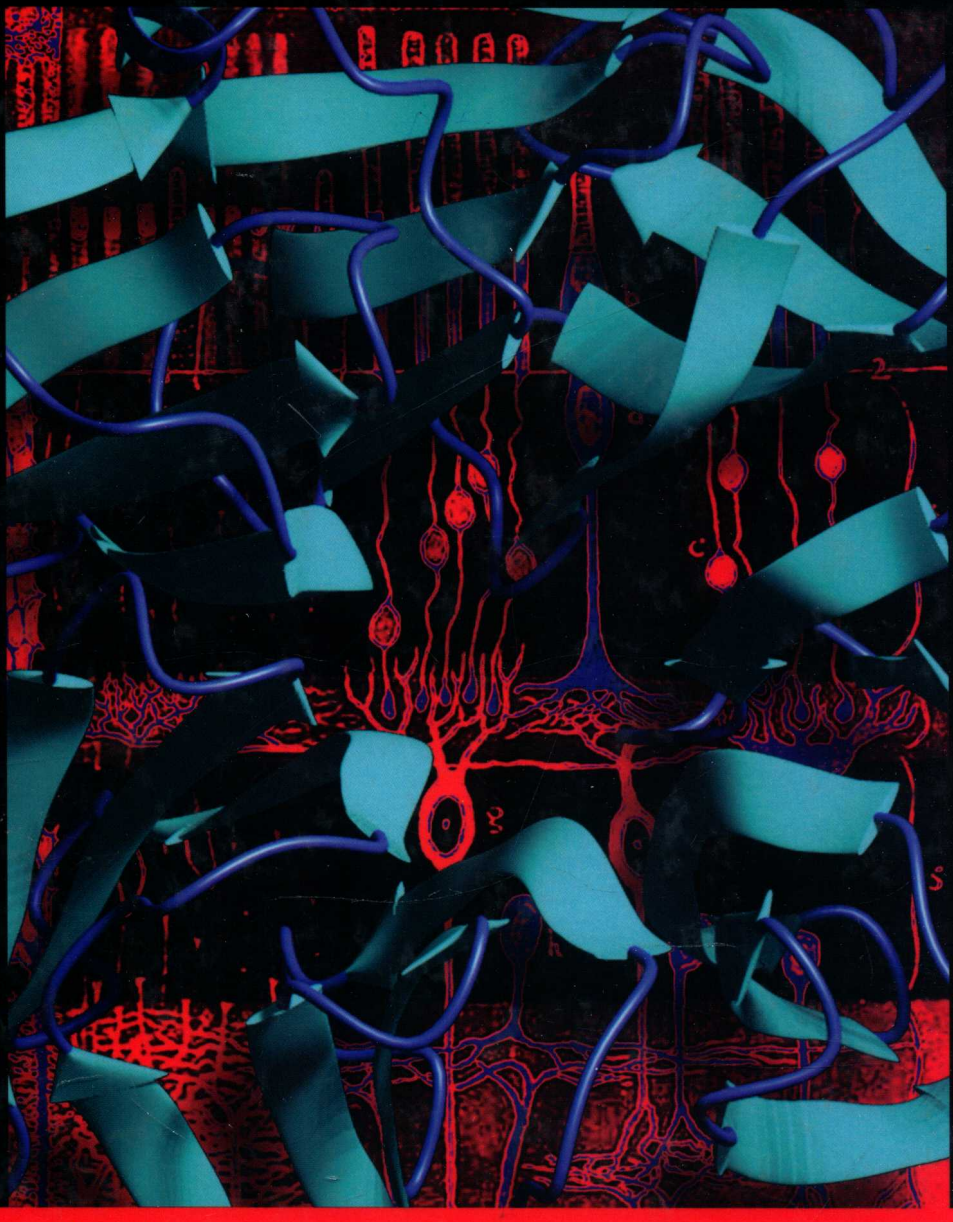
SIGNAL TRANSDUCTION IN THE RETINA



Edited by
Steven J. Fliesler & Oleg G. Kisselev

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SIGNAL TRANSDUCTION IN THE RETINA

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Joseph Eichberg, Jr., Series Editor

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The Editors dedicate this volume
to Dr. Paul A. Hargrave, in honor of his numerous
and significant contributions to the field of vision science,
particularly in regard to the biochemistry and structure
of rhodopsin and the current understanding of the
phototransduction cascade in retinal rod cells.

Series Preface

The concept of signal transduction at the cellular level is now established as a cornerstone of the biological sciences. Cells sense and react to environmental cues by means of a vast panoply of signaling pathways and cascades. While the steady accretion of knowledge regarding signal transduction mechanisms is continuing to add layers of complexity, this greater depth of understanding has also provided remarkable insights into how healthy cells respond to extracellular and intracellular stimuli and how these responses can malfunction in many disease states.

Central to advances in unraveling signal transduction is the development of new methods and refinement of existing ones. Progress in the field relies upon an integrated approach that utilizes techniques drawn from cell and molecular biology, biochemistry, genetics, immunology, and computational biology. The overall aim of this series is to collate and continually update the wealth of methodology now available for research into many aspects of signal transduction. Each volume is assembled by one or more editors who are leaders in their specialty. Their guiding principle is to recruit knowledgeable authors who will present procedures and protocols in a critical yet reader-friendly format. Our goal is to assure that each volume will be of maximum practical value to a broad audience, including students, seasoned investigators, and researchers who are new to the field.

The retina has long been a favorite system for the study of signal transduction mechanisms because of its accessibility and the relative abundance of several of the components of the visual phototransduction pathway. Its investigation has yielded valuable insights into the molecular transformation that accompany the transmission of light signals. The editors of this volume have brought together a distinguished group of authors who build on existing knowledge to describe the latest methodological innovations for the exploration of phototransduction at the level of individual molecules. While the chief focus is on vertebrate photo-transduction, aspects of non-visual and invertebrate phototransduction are also covered. Additional chapters deal with techniques used to investigate the rapidly developing area of insulin-mediated signaling in the retina, and with approaches to elucidate the role of signal transduction in the development of retina and supporting structures. A particularly attractive feature is the inclusion of protocols that provide detailed guidance in applying a variety of experimental methods to study retinal signal transduction. Without question, this volume will be a valuable reference to all investigators who are active or interested in this field.

Joseph Eichberg, Ph.D.
Advisory Editor for the Series

Preface

In the current "postgenomic era," there is increasing recognition of the need for integrated approaches to study and understand complex biological systems and signaling networks. The retina—an anatomically and functionally unique part of the central nervous system, responsible for the detection and initial processing of visual information—is illustrative of this. An integrated knowledge of the biochemistry, cell biology, physiology, and physics of phototransduction, as well as postphotoreceptor visual transduction processes, has evolved over the past century, with the finer details becoming apparent particularly within the past decade. The retina is an extremely useful biological system amenable to experimental manipulation in vivo as well as in vitro, affording an accessible model with which to understand individual cellular signaling systems down to the level of molecular interactions at atomic resolution, as well as more complex issues of pathway regulation and the integration of signaling networks that impact cellular and tissue responses, ultimately resulting in visual perception.

The present volume, comprised of fifteen chapters in six sections, brings together a number of internationally recognized authorities in disciplines pertinent to the study of signal transduction in the retina. Each chapter presents a brief overview of the background and current state of knowledge in a particular area relevant to the broader topic of retinal signal transduction, along with detailed information regarding specific methodology for obtaining the primary data necessary to understand the molecular and cellular processes being examined. Because more is known about the rhodopsin-based phototransduction pathway in vertebrate retinal rod cells than in almost any other biological system, and this dominates signaling processes in the retina, a substantial portion of this volume is devoted to that topic. In addition, a diversity of other signaling mechanisms and systems are covered, affording the reader a resource for evaluating the similarities and differences between these systems and the specific research strategies employed for studying them.

Section 1 deals with the molecular mechanisms of vertebrate phototransduction, dissecting the major components of the phototransduction cascade in rod cells and proteins involved in its regulation. The chapters in this section emphasize the breadth of knowledge accumulated in the past decade, especially with regard to determination of the molecular structure of phototransduction cascade components at atomic resolution, as well as the use of transgenic strategies. State-of-the-art approaches for the study of molecular interactions in multiprotein complexes, as well as novel cell-based strategies aimed at understanding the mechanisms of signal shut-off and light adaptation, are presented.

Section 2 focuses on the more recently emerging field of nonvisual phototransduction. Methods for assessing the roles of melanopsin in regulation of the circadian clock and in adaptive photoresponses are described.

Section 3 provides a chapter devoted to essential methods for studying phototransduction in the invertebrate retina, using *Drosophila* as the biological system

of choice. Thus, the reader will be able to compare and contrast the juxtaposing processes of visual signaling in vertebrates versus invertebrates.

Section 4 focuses on experimental studies of insulin-based signaling in the retina, both in the outer retina (photoreceptors, per se) as well as inner retinal cells. In addition, insulin receptor structure and ligand-binding specificity as well as mechanisms of downstream signaling are described.

Section 5 presents current methodological approaches relevant to retinal development, including cellular signaling in retinal progenitor cells, and cell–cell communications in developing retina. Because neovascularization is considered an increasingly important factor in various human degenerative retina diseases, particularly those that accompany diabetes and aging, this section also addresses experimental approaches for studying vascular homeostasis.

Section 6 deals with recent developments in the field of lipid-derived mediators, particularly neuroprotectins and the participation of the retinal pigment epithelium in neuronal survival in the retina.

Now in the twenty-first century, we are just beginning to understand the enormous diversity and complexity of signaling processes in the retina. The methodologies and experimental approaches described in this volume have already yielded key fundamental information regarding the cellular and molecular mechanisms that underlie normal retinal physiology. In addition, they have the potential to provide new clues toward elucidating the mechanisms involved in retinal disease processes as well as the development of novel therapeutic approaches for preventing, arresting, and modulating those disease processes and promoting cellular survival and retention of function.

Steven J. Fliesler
Oleg G. Kisselev

Editors

Steven J. Fliesler is a professor and director of research, Department of Ophthalmology (Saint Louis University Eye Institute), as well as a professor in the Department of Pharmacological and Physiological Science at Saint Louis University School of Medicine, in St. Louis, Missouri. He earned a B.A. degree in biochemistry, with a minor in chemistry, from the University of California–Berkeley in 1973, and a Ph.D. degree in biochemistry from Rice University (Houston, Texas) in 1980. Following a three-year postdoctoral research fellowship on retinal lipid metabolism at the Cullen Eye Institute, Baylor College of Medicine (Houston, Texas), Fliesler pursued studies funded by the National Institutes of Health (NIH) on glycoprotein metabolism and photoreceptor membrane assembly in the retina as a research assistant professor at the Cullen Eye Institute. In 1985, Fliesler moved to the Bascom Palmer Eye Institute, with a joint appointment as an assistant professor in the Department of Biochemistry and Molecular Biology and in the Program in Neuroscience at the University of Miami School of Medicine, where he continued his studies on glycoprotein metabolism in the retina. In 1988, he was appointed associate professor at the Bethesda Eye Institute (now Saint Louis University Eye Institute), with a secondary appointment as associate professor in the E.A. Doisy Department of Biochemistry and Molecular Biology and the Cell and Molecular Biology Graduate Program at Saint Louis University School of Medicine (St. Louis, Missouri). Subsequently, Fliesler was promoted (in 1994) to professor in the Department of Ophthalmology, with a secondary appointment (in 2000) as professor in the Department of Pharmacological and Physiological Science at Saint Louis University School of Medicine.

Fliesler's research program encompasses studies on the relationship between cellular metabolism and the establishment and preservation of retinal structure and function, especially in regard to retinal rod photoreceptor cells. A major focus of his research has been cholesterol metabolism and dyslipidemias caused by inborn errors in cholesterol metabolism. In addition, he has a long-standing interest in animal models of human hereditary retinal degenerations, particular those involving defective membrane transport and assembly in retinal photoreceptor cells. Fliesler is the author or coauthor of over 90 peer-reviewed publications, book chapters, and review articles dealing largely with retinal cell biology and glycoprotein and lipid metabolism, and has delivered more than 150 presentations at national and international scientific meetings, colleges and universities, and specialty scientific and biomedical symposia. In addition to editing this volume, Fliesler is the editor of *Sterols and Oxysterols: Chemistry, Biology and Pathobiology*, a multiauthor volume in the "Recent Research Developments in Biochemistry" series, published by Research Signpost (Kerala, India) in 2002. Fliesler is an editorial board member, Retina and Choroid Section editor, and "Focus on Molecules" feature editor for *Experimental Eye Research*, and also serves on the editorial board of the *Journal of Lipid Research*. In addition, he also serves regularly as a reviewer for a variety of federal, state, and private grant-funding agencies, including the National Eye Institute, the Foundation

Fighting Blindness, and Fight for Sight. Fliesler is the recipient of numerous research grant awards, both from federal and private funding agencies, including the National Institutes of Health, the Foundation Fighting Blindness, the March of Dimes Foundation, and Research to Prevent Blindness. In 2007, he was selected as the recipient of a Senior Scientific Investigator Award from Research to Prevent Blindness.

Oleg G. Kisselev is currently an associate professor in the Department of Ophthalmology (Saint Louis University Eye Institute), with a secondary faculty appointment in the Edward A. Doisy Department of Biochemistry and Molecular Biology at Saint Louis University School of Medicine. Kisselev obtained his undergraduate and Ph.D. degrees in biochemistry at Lomonosov Moscow State University, Moscow. In 1992, he emigrated to the United States to pursue postdoctoral training in the laboratory of N. Gautam in the Department of Anesthesiology at Washington University School of Medicine in St. Louis. He later became an American Heart Association Fellow and, in 1997, he received his first faculty appointment as an instructor in that department with a secondary appointment in the Center for Molecular Design headed by Professor Garland R. Marshall at Washington University. In 1999, Kisselev established his own independent research program upon accepting a faculty position in the Department of Ophthalmology (Saint Louis University Eye Institute) of Saint Louis University School of Medicine. He was promoted to his current rank of associate professor in 2007.

Kisselev's research interests are in the biology of sensory signaling utilizing universal mechanisms of transmembrane signal transduction mediated by heterotrimeric GTP-binding proteins, and G-protein-coupled cell surface receptors. He has made seminal contributions to the current state of knowledge regarding the role of individual G-protein subunits in determining the specificity of G-protein signaling and the mechanism of receptor-catalyzed G-protein activation, especially in the vertebrate visual system. His studies of interactions between phototransduction proteins using high-resolution nuclear magnetic resonance (NMR) methods have helped to elucidate the dynamics of the phototransduction machinery at atomic resolution, and have provided essential refinements to the mechanism of visual signal transduction. Kisselev is an author of more than 25 scientific publications including reviews and book chapters dealing with the biochemistry and structural biology of G-proteins. He has received funding for his research program from the National Institutes of Health and the American Heart Association. In addition, he received a William and Mary Greve Scholar Award in 2002 from Research to Prevent Blindness.

List of Contributors

Lingling An
Department of Statistics
Purdue University
West Lafayette, Indiana

Robert E. Anderson
Departments of Ophthalmology and Cell Biology, and
Dean A. McGee Eye Institute
Health Sciences Center
University of Oklahoma
Oklahoma City, Oklahoma

Nikolai O. Artemyev
Department of Molecular Physiology and Biophysics
College of Medicine
University of Iowa
Iowa City, Iowa

Nicolas G. Bazan
Neuroscience Center of Excellence and Department of Ophthalmology
Health Sciences Center
School of Medicine
Louisiana State University
New Orleans, Louisiana

Shawn Beug
Molecular Medicine Program and Vision Program
Ottawa Health Research Institute and Department of Biochemistry, Microbiology and Immunology
University of Ottawa
Ottawa, Ontario, Canada

Terri DiMaio
Department of Ophthalmology and Visual Sciences
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Rebecca W. Doerge
Department of Statistics
Purdue University
West Lafayette, Indiana

Patrice E. Fort
Departments of Ophthalmology and Cellular and Molecular Physiology
College of Medicine
Pennsylvania State University
Hershey, Pennsylvania

Thomas W. Gardner
Departments of Ophthalmology and Cellular and Molecular Physiology
College of Medicine
Pennsylvania State University
Hershey, Pennsylvania

Eugenia V. Gurevich
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Vsevolod V. Gurevich
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Heidi E. Hamm
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Susan M. Hanson
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Eric L. Harness
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Byron Hartman
Department of Biological Structure
School of Medicine
University of Washington
Seattle, Washington

Susan Hayes
Department of Biological Structure
School of Medicine
University of Washington
Seattle, Washington

Andreas Helten
Biochemistry Group
Department of Biology and
Environmental Sciences
Carl von Ossietzky University
Oldenburg, Germany

Song Hong
Neuroscience Center of Excellence and
Department of Ophthalmology
School of Medicine
Health Sciences Center
Louisiana State University
New Orleans, Louisiana

Fannie Jackson
Neuroscience Center of Excellence and
Department of Ophthalmology
School of Medicine
Health Sciences Center
Louisiana State University
New Orleans, Louisiana

Kacee Jones
Regulatory Biology Laboratory
The Salk Institute of Biological
Sciences
La Jolla, California

Eunju Kim
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Oleg G. Kisselev
Departments of Ophthalmology and
Biochemistry and Molecular Biology
School of Medicine
Saint Louis University
Saint Louis, Missouri

Junko Kitamoto
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Karl-Wilhelm Koch
Biochemistry Group
Department of Biology and
Environmental Sciences
Carl von Ossietzky University
Oldenburg, Germany

Shuji Kondo
Department of Pediatrics
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Hiep Le
Regulatory Biology Laboratory
The Salk Institute of Biological Sciences
La Jolla, California

Janis Lem
Molecular Cardiology Research Institute and Department of Ophthalmology
Tufts-New England Medical Center
Tufts University
Boston, Massachusetts
Sackler School of Graduate Biomedical Sciences
School of Medicine

Hung-Tat Leung
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Guohua Li
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Mandy K. Losiewicz
Departments of Ophthalmology and Cellular and Molecular Physiology
College of Medicine
Pennsylvania State University
Hershey, Pennsylvania

Yan Lu
Neuroscience Center of Excellence and Department of Ophthalmology
School of Medicine
Louisiana State University
New Orleans, Louisiana

Victor L. Marcheselli
Neuroscience Center of Excellence and Department of Ophthalmology
School of Medicine
Louisiana State University
New Orleans, Louisiana

Brian McNeill
Ottawa Health Research Institute and Department of Biochemistry, Microbiology, and Immunology
University of Ottawa
Ottawa, Ontario
Canada

Hakim Muradov
Department of Molecular Physiology and Biophysics
College of Medicine
University of Iowa
Iowa City, Iowa

Michael Natochin
Department of Molecular Physiology and Biophysics
College of Medicine
University of Iowa
Iowa City, Iowa

Surendra Kuman Nayak
Genomics Institute of Novartis Research Foundation
San Diego, California

Branden Nelson
Department of Biological Structure
School of Medicine
University of Washington
Seattle, Washington

William M. Oldham
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

William L. Pak
Department of Biological Sciences
School of Medicine
Purdue University
West Lafayette, Indiana

Satchidananda Panda
Regulatory Biology Laboratory
The Salk Institute of Biological Sciences
La Jolla, California

Victoria Piamonte
Genomics Institute of Novartis
Research Foundation
San Diego, California

Anita M. Preininger
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Raju V. S. Rajala
Departments of Ophthalmology and
Cell Biology, and Dean A. McGee
Eye Institute
Health Sciences Center
University of Oklahoma
Oklahoma City, Oklahoma

Thomas Reh
Department of Biological Structure
School of Medicine
University of Washington
Seattle, Washington

Kibibi Rwayitare
Molecular Cardiology Research
Institute and Department of
Ophthalmology
Tufts-New England Medical Center
School of Medicine
Tufts University
Boston, Massachusetts

Elizabeth A. Scheef
Department of Ophthalmology and
Visual Sciences
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Nader Sheibani
Departments of Ophthalmology and
Visual Sciences and Pharmacology
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Ravi S. J. Singh
Departments of Ophthalmology and
Cellular and Molecular Physiology
College of Medicine
Pennsylvania State University
Hershey, Pennsylvania

Christine M. Sorenson
Department of Pediatrics
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Nobushige Tanaka
Regulatory Biology Laboratory
The Salk Institute of Biological
Sciences
La Jolla, California

Yixin Tang
Department of Ophthalmology and
Visual Sciences
School of Medicine and Public Health
University of Wisconsin
Madison, Wisconsin

Julie Tseng-Crank
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Sergey A. Vishnivetskiy
Department of Pharmacology
School of Medicine
Vanderbilt University
Nashville, Tennessee

Dana Wall
Ottawa Health Research Institute
and Department of Biochemistry,
Microbiology, and Immunology
University of Ottawa
Ottawa, Ontario, Canada

Valerie A. Wallace
Ottawa Health Research Institute
and Department of Biochemistry,
Microbiology, and Immunology
University of Ottawa
Ottawa, Ontario, Canada

Qiong Wang
Department of Biochemistry and
Molecular Biology
College of Medicine
Baylor University
Houston, Texas

Theodore G. Wensel
Department of Biochemistry and
Molecular Biology
College of Medicine
Baylor University
Houston, Texas

Sowmya V. Yelamanchili
Regulatory Biology Laboratory
The Salk Institute of Biological
Sciences
La Jolla, California

Ying Zhou
Department of Biological Sciences
Purdue University
West Lafayette, Indiana

Quansheng Zhu
Regulatory Biology Laboratory
The Salk Institute of Biological
Sciences
La Jolla, California

Contents