Springer Series in Vision Research

Kirill A. Martemyanov
Alapakkam P. Sampath *Editors*

G Protein Signaling Mechanisms in the Retina

Springer Series in Vision Research

Volume 3

Editors
N. Justin Marshall
Brisbane, Australia

Shaun P. Collin
Crawley, Australia

The Springer Series in Vision Research is a comprehensive update and overview of cutting edge vision research, exploring, in depth, current breakthroughs at a conceptual level. It details the whole visual system, from molecular processes to anatomy, physiology and behavior and covers both invertebrate and vertebrate organisms from terrestrial and aquatic habitats. Each book in the Series is aimed at all individuals with interests in vision including advanced graduate students, post-doctoral researchers, established vision scientists and clinical investigators. The series editors are N. Justin Marshall, Queensland Brain Institute, The University of Queensland, Australia and Shaun P. Collin, Neuroecology Group within the School of Animal Biology and the Oceans Institute at the University of Western Australia.

More information about this series at http://www.springer.com/series/10633

Kirill A. Martemyanov • Alapakkam P. Sampath
Editors

G Protein Signaling Mechanisms in the Retina

Editors
Kirill A. Martemyanov
The Scripps Research Institute
Jupiter
FL
USA

Alapakkam P. Sampath
Jules Stein Eye Institute
University of California, Los Angeles
Los Angeles
CA
USA

ISBN 978-1-4939-1217-9 ISBN 978-1-4939-1218-6 (eBook)
DOI 10.1007/978-1-4939-1218-6
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014945638

© Springer Science+Business Media New York 2014
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

1 Introduction: G-Protein Signaling in the Retina 1
Kirill A. Martemyanov and Alapakkam P. Sampath

Part I Phototransduction in Rods and Cones

2 G Protein Deactivation Mechanisms in Vertebrate Phototransduction 9
Ching-Kang Chen and Hung-Ya Tu

3 Signaling by Rod and Cone Photoreceptors: Opsin Properties, G-protein Assembly, and Mechanisms of Activation 23
Alexander V. Kolesnikov, Oleg G. Kisselev and Vladimir J. Kefalov

4 G-Protein–Effector Coupling in the Vertebrate Phototransduction Cascade 49
Nikolai O. Artemyev

Part II Inner Retinal GPCR Signaling Pathways

5 Interdependence Among Members of the mGluR6 G-protein Mediated Signalplex of Retinal Depolarizing Bipolar Cells 67
Ronald G. Gregg, Thomas A. Ray, Nazarul Hasan, Maureen A. McCall and Neal S. Peachey

6 Mechanistic Basis for G Protein Function in ON Bipolar Cells 81
Noga Vardi and Anuradha Dhingra

7 Modulation of TRPM1 and the mGluR6 Cascade in ON Bipolar Cells 99
Scott Nawy

8 The Role of Dopamine in Fine-Tuning Cone- and Rod-Driven Vision 121
Rolf Herrmann and Vadim Y. Arshavsky

9 Regulation of Electrical Synaptic Plasticity in the Retina by G-Protein-Coupled Receptors 143
John O'Brien

Part III Signaling by Photosensitive Ganglion Cells

10 The Functional Properties of the G Protein-Coupled Receptor Melanopsin in Intrinsically Photosensitive Retinal Ganglion Cells 173
Alan C. Rupp and Samer Hattar

Index 197

Contributors

Vadim Y. Arshavsky Department of Ophthalmology and Pharmacology, Albert Eye Research Institute, Duke University Medical Center, Durham, NC, USA

Nikolai O. Artemyev Department of Molecular Physiology and Biophysics Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA, USA

Ching-Kang Chen Departments of Ophthalmology, Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

Anuradha Dhingra Department of Neuroscience, University of Pennsylvania, Philadelphia, PA, USA

Ronald G. Gregg Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, KY, USA

Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA

Nazarul Hasan Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, KY, USA

Samer Hattar Department of Biology, Johns Hopkins University, Baltimore, MD, USA

Rolf Herrmann Department of Ophthalmology and Cell Biology, Neurobiology and Anatomy, The Eye Institute, Medical College of Wisconsin, Milwaukee, WI, USA

Vladimir J. Kefalov Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

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

Alexander V. Kolesnikov Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

Kirill A. Martemyanov Department of Neuroscience, The Scripps Research Institute, Jupiter, FL, USA

Maureen A. McCall Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA

Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, USA

Scott Nawy Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, NY, USA

Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA

John O'Brien The Richard S. Ruiz, M.D. Department of Ophthalmology and Visual Science and The Graduate School of Biomedical Sciences, The University of Texas Houston Medical School, Houston, TX, USA

Neal S. Peachey Cole Eye Institute, Cleveland Clinic, Cleveland, OH, USA

Louis Stokes Cleveland VA Medical Center, Cleveland, OH, USA

Department of Ophthalmology, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA

Thomas A. Ray Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, KY, USA

Alan C. Rupp Department of Biology, Johns Hopkins University, Baltimore, MD, USA

Alapakkam P. Sampath Department of Ophthalmology, Jules Stein Eye Institute, University of California, Los Angeles, CA, USA

Hung-Ya Tu Departments of Ophthalmology, Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

Institute of Molecular Medicine, National Tsing-Hua University, Hsinchu, Taiwan, Republic of China

Noga Vardi Department of Neuroscience, University of Pennsylvania, Philadelphia, PA, USA

Chapter 1
Introduction: G-Protein Signaling in the Retina

Kirill A. Martemyanov and Alapakkam P. Sampath

Abstract Mammalian organisms are composed of a multitude of cells with unique anatomical and physiological specifications that perform a vast variety of functions. The survival of mammals is hinged on seamless integration of cells and coordination of their processes, within a particular structure or organ, or between organs. Intracellular signaling pathways play an indispensable role in this process. The typical architecture for such pathways includes cell surface receptors for sensing molecules or physical stimuli and the intracellular machinery for converting their presence into changes in biochemical activity that allows the organism to exploit this information.

Mammalian organisms are composed of a multitude of cells with unique anatomical and physiological specifications that perform a vast variety of functions. The survival of mammals is hinged on seamless integration of cells and coordination of their processes, within a particular structure or organ, or between organs. Intracellular signaling pathways play an indispensable role in this process. The typical architecture for such pathways includes cell surface receptors for sensing molecules or physical stimuli and the intracellular machinery for converting their presence into changes in biochemical activity that allows the organism to exploit this information.

The largest family of cell surface receptors is formed by the G-protein-coupled receptors (GPCRs), whose members account for approximately 3–4 % of mammalian genomes. These receptors all share a common structure of seven transmembrane domains and their activity on the cell surface leads to signaling within the cell. Indeed, G proteins (or guanosine triphosphate (GTP)-binding proteins) are the

K. A. Martemyanov (✉)
Department of Neuroscience, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458, USA
e-mail: kirill@scripps.edu

A. P. Sampath
Department of Ophthalmology, Jules Stein Eye Institute, University of California, Los Angeles, 100 Stein Plaza, Los Angeles, CA 90095-7000, USA
e-mail: asampath@jsei.ucla.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,* Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_1

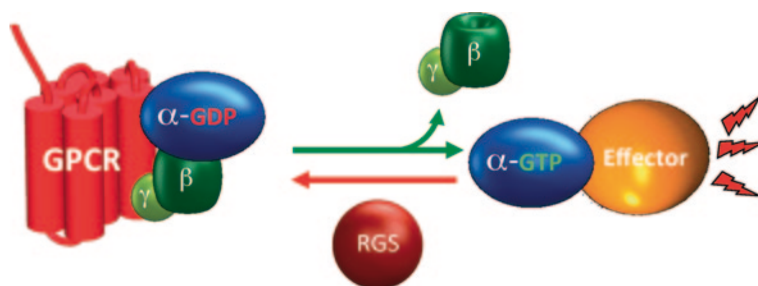

Fig. 1.1 Key reactions in G-protein signaling

targets of GPCRs, which serve to couple the receptor activity with effector molecules, a collective term for a vast number of second messenger enzymes, kinases, ion channels, and transcription factors that influence cellular activity. In the prototypic sequence of events, agonist-bound GPCRs activate G proteins by catalyzing their guanosine diphosphate (GDP)/GTP exchange on the Gα subunit. Upon GTP binding, G proteins dissociate into Gα and Gβγ subunits and both Gα-GTP and free Gβγ subunits are able to activate or inhibit downstream effector molecules (Fig. 1.1). Termination of the response requires inactivation of both the GPCR, which can be quenched by phosphorylation and the binding of arrestin proteins, and the G protein, which is achieved when the Gα subunit hydrolyzes GTP, and the inactive GDP-bound form reassociates with the βγ subunits.

GPCRs mediate a wide range of functions, ranging from sensory transduction to hormone action, in a large range of tissues. These receptors are major targets for therapeutics, and the great importance of their associated signal transduction pathways to biology is highlighted if one simply peruses the list of Nobel Prize winners in *Chemistry*, or in *Physiology or Medicine*. Indeed, Nobel Prizes have been awarded for a number of the studies of GPCRs: the characterization of rhodopsin by Granit, Hartline, and Wald in 1957; the identification of odorant receptor proteins by Axel and Buck in 2004; for structural studies of β-adrenergic receptors by Kobilka and Lefkowitz in 2012; the identification of G proteins by Gilman and Rodbell in 1994; and classical studies of second messenger action by Sutherland in 1971. In addition, many other Nobel Prizes have been awarded for related studies of signal transduction and ion channel function.

Light is the main form of energy that fuels life on Earth. The ability to interact with it is built into almost every organism. The gateway for light reception in mammals is a neuronal structure called the retina. Perhaps not surprisingly, GPCR signaling pathways are indispensable for receiving and processing light signals in the retina. Historically, the first GPCR pathway understood at a high level of molecular detail was the phototransduction cascade of vertebrate rod and cone photoreceptors. Photoreceptor cells display significant advantages for the study of G-protein-coupled signaling pathways. Firstly, the cascade components are isolated in a privileged compartment, perfectly accessible for physiological experiments and

for high-throughput biochemistry. Secondly, the stimulus (light) can be controlled with precision, allowing the number of activated GPCRs, or incident photons, to be estimated and the resulting activity to be measured. Furthermore, a significant portion of the cell's dynamic range encodes a linear representation of the number of photons absorbed, allowing linear systems and quantitative methods to be used. Thirdly, most components of the system are expressed only in the photoreceptor outer segments and nowhere else in the body, allowing targeted genetics with surprisingly little dosage compensation or developmental abnormalities. Due to these advantages, technological innovations in biochemistry and physiology in the 1970s significantly advanced studies of retinal phototransduction. Classical work by Baylor, Lamb, and Yau [13] suggested in toad rod photoreceptors that slow light-evoked responses could not be explained by direct gating of ion channels by the stimulus, instead suggesting that the responses are produced by a sequence of chemical reactions and the buildup of some messenger molecule. Among the leading candidates for this putative messenger were Ca^{2+} and cyclic guanosine monophosphate (cGMP). In the early 1980s, the biochemistry of rhodopsin → G protein (transducin) → effector (cGMP phosphodiesterase) was established [3, 5], but it was not until 1985 that seminal physiological work showed that cGMP indeed directly gated transduction channels [2, 12]. Cone photoreceptors have since been shown to operate with similar cGMP-gated channels [4].

In the ensuing decades, the mechanistic basis for the photoresponse has been studied quantitatively and has served as a general template for G-protein signaling. In the retina, the signaling cascade in the dendrites of ON-bipolar cells (BCs), which receive direct glutamatergic inputs from photoreceptors, provides a vivid example. Following the physiological demonstration that glutamate analogue L-AP4/APB suppresses the activity of ON-BCs [11], it was hypothesized that G proteins might play a role in signal transmission. Indeed, subsequent work established that ON-BC responses required GTP hydrolysis [9]. Additionally, the glutamate receptor mGluR6 was shown to be the GPCR with selectivity for L-AP4/APB [8]. In the following two decades, many additional players were implicated in this signaling cascade including the G protein ($G\alpha_o$) [1], the transduction channel (TRPM1) [6, 7, 10], and an array of regulatory elements that coordinate transmission of signal between photoreceptors and ON-BCs. Despite overall similarity, the phototransduction cascade of photoreceptors and the signaling pathway of the ON-BC display notable differences in their temporal characteristics, the spatial organization of components, and the properties of the effector ion channels. These differences place key constraints on the properties of these individual pathways. Contrasting these properties, while taking into account physiological needs and specializations of individual cells, has and will continue to generate significant insight into the overall understanding of the architecture of GPCR pathways.

In recent years, other G-protein cascades in the retina have also become better characterized. The logistical advantages of working with retinal tissue can also be leveraged to establish a common logic for how these pathways operate in vivo. For instance, the catecholamine dopamine has been shown to provide slow modulation of retinal sensitivity via regulating electrical coupling between neurons via

gap junctions and by adjusting response sensitivity through the control of neuronal excitability. Additionally, these mechanisms appear to guide another emerging G-protein cascade in the retina, melanopsin-based signaling in intrinsically photosensitive retinal ganglion cells (ipRGCs). Directly activated by light, this GPCR pathway is responsible for circadian light entrainment and the pupillary reflex. Once the organizational principles and logistics of signal transduction in these cascades are established, perhaps the next frontier would be to determine how these different cascades are coordinated.

We have learned a great deal about the organization and function of GPCR pathways, and the role that they play in vision. The information gained from these studies has provided, and will continue to provide, critical insights to further our understanding of complex GPCR pathways in the central nervous and endocrine systems. In essence, the retina is the first "optogenetically" driven circuit with clearly understood physiology. Thus, studying GPCR-driven pathways in the retina will likely guide the interpretation of optogenetic experiments, which are increasingly utilized to study central circuits.

The goal of this volume is to summarize our current understanding of the organizational principles of GPCR pathways, using insights derived from the study of the retina. We have highlighted several G-protein signaling cascades, including phototransduction, ON-BC signaling, dopaminergic pathways, and ipRGC signaling. This volume will generally follow the path of signal flow through the retina. The first chapters will focus on the elucidation of the phototransduction cascade of rod and cone photoreceptors. These chapters will begin with a discussion of the activation and shutoff steps of phototransduction, with a treatment on the rate-limiting reaction for shutoff of the light response (Chap. 1). This will be followed by a comparison of rod and cone visual pigments and G proteins, with particular focus on differences in their physiological properties (Chap. 2). A description of what is understood about interactions between the G protein and the effector phosphodiesterase is provided in Chap. 3. The subsequent chapters will delve into our current understanding of the mechanisms of synaptic information encoding by retinal ON-BCs, where the GPCR mGluR6 plays a fundamental role. Chapters in this section will examine the physiological features of the ON-BC response (Chap. 4), an analysis of the components of the mGluR6 cascade (Chap. 5), and the macromolecular organization of the mGluR6 signaling complex (Chap. 6). Two additional chapters will evaluate the role of dopamine in electrical coupling between retinal neurons (Chap. 7), and its role in setting the excitability of rod BCs (Chap. 8). The final chapter (Chap. 9) will be focused on the output neurons of the inner retina, specifically the ganglion cells, where the components of the emerging GPCR melanopsin cascade in intrinsically photosensitive ganglion cells will be detailed. Collectively, these signaling pathways allow the retina to represent visual space over a wide range of light intensities and to synchronize its function to the day/night cycle. We hope that the rigorous study of these mechanisms will provide more general insights into G-protein signaling.

References

1. Dhingra A, Lyubarsky A, Jiang MS, Pugh EN Jr., Birnbaumer L, Sterling P, Vardi N (2000) The light response of ON bipolar neurons requires Gao. J Neurosci 20:9053–9058
2. Fesenko EE, Kolesnikov, SS, Lyubarsky AL (1985) Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature 313:310–313
3. Fung BB-K, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc Natl Acad Sci U S A 78:152–156
4. Haynes LW, Yau K-W (1990) Single-channel measurement from the cyclic GMP-activated conductance of catfish retinal cones. J Physiol 429:451–481
5. Hurley JB, Stryer L (1982) Purification and characterization of the gamma regulatory subunit of the cGMP phosphodiesterase from retinal rod outer segments. J Biol Chem 257:11094–11099
6. Koike C, Obara T, Uriu Y, Numata T, Sanuki R, Miyata K, Koyasu T, Ueno S, Funabiki K, Tani A et al. (2010) TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. Proc Natl Acad Sci U S A 107:332–337
7. Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS, Duvoisin RM, Brown RL (2009) TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proc Natl Acad Sci U S A 106:19174–19178
8. Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N, Nakanishi S (1993) Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. J Biol Chem 268:11868–11873
9. Nawy S, Jahr CE (1990) Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. Nature 346:269–271
10. Shen Y, Heimel JA, Kamermans M, Peachey NS, Gregg RG, Nawy S (2009) A transient receptor potential-like channel mediates synaptic transmission in rod bipolar cells. J Neurosci 29:6088–6093
11. Slaughter MM, Miller RF (1983) Bipolar cells in the mudpuppy retina use an excitatory amino acid neurotransmitter. Nature 303:537–538
12. Yau K-W, Nakatani K (1985) Light-suppressible, cyclic GMP-sensitive conductance in the plasma membrane of a truncated rod outer segment. Nature 317:252–255
13. Yau KW, Lamb TD, Baylor DA (1977) Light-induced fluctuations in membrane current of single toad rod outer segments. Nature 269:78–80

Part I
Phototransduction in Rods and Cones

Chapter 2
G Protein Deactivation Mechanisms in Vertebrate Phototransduction

Ching-Kang Chen and Hung-Ya Tu

Abstract Heterotrimeric G proteins are widely used in nature to facilitate cellular responses to extracellular stimuli. In humans, these G proteins mediate vision and other senses, modulate neurotransmission, and are required for hormonal actions. The signaling system involves three groups of molecules, namely, the receptors with heptahelical transmembrane motifs, the trimeric G proteins themselves, and the effectors through which G proteins alter cellular homeostasis. Advances in genome sciences have revealed the full complement of this system in multiple species, and the current challenges are to elucidate which, when, where, and how each component is used. The field of phototransduction has historically provided unrivaled details in describing general principles of G-protein signaling. This chapter intends to cover the reactions that dominate the rate of phototransduction recovery in rod and cone photoreceptors.

Phototransduction Mechanisms

Vertebrate retinal photoreceptors are terminally differentiated cells with organized subcellular compartments tailored for distinct functions [5, 68]. Other than organelles common to eukaryotic cells, a unique compartment of photoreceptors that allows its identification is the outer segment. This structure contains tightly packed membrane discs enclosed in plasma membranes in rods and an invaginated, sometimes tapered, plasma membrane in cones. The outer segment links to the inner segment through a delicate connecting cilium, a microtubule-based structure broken off easily in solution by mechanical means. This allows the membrane-rich, and hence buoyant, outer segment to "float" during centrifugation for rapid isolation [75]. The

C.-K. Chen (✉) · H.-Y. Tu
Departments of Ophthalmology, Biochemistry and Molecular Biology,
Baylor College of Medicine, 6550 Fannin Street, Rm NC305, Houston, TX 77030, USA
e-mail: Ching-Kang.Chen@bcm.edu

H.-Y. Tu
Institute of Molecular Medicine, National Tsing-Hua University, No. 101, Section 2,
Kuang-Fu Road, 30013 Hsinchu, Taiwan, Republic of China
e-mail: d9680510@oz.nthu.edu.tw

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_2

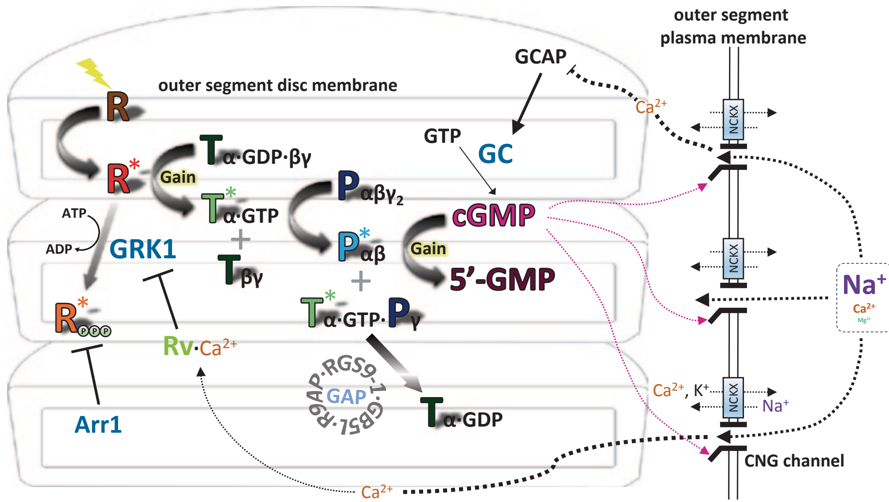

Fig. 2.1 Phototransduction activation and recovery in a vertebrate rod. Photon absorption by rhodopsin (*R*) leads to the formation of metarhodopsin II (*R**), which in turn catalyzes guanine nucleotide exchange on α subunit of the heterotrimeric transducin. Activated transducin dissociates into $T^{*}\alpha{\cdot}GTP$ and $T\beta\gamma$ moieties, with the former ($T^{*}\alpha{\cdot}GTP$) interacting with the inhibitory γ subunits of phosphodiesterase (*PDE*; $P_{\alpha\beta\gamma2}$) to disinhibit the catalytic subunits ($P^{*}_{\alpha\beta}$) and allow free cGMP to be rapidly hydrolyzed to *5′-GMP*. The decline of free *cGMP* leads to the closure of cyclic-nucleotide-gated (*CNG*) channels and membrane hyperpolarization. While *CNG channels* are close and Ca^{2+} entry is blocked, the Na/Ca, K exchanger (*NCKX*) continues to extrude Ca^{2+}, leading to a decline in intracellular free Ca^{2+} concentration. This reduction in Ca^{2+} is sensed by two calcium-binding proteins, recoverin (*Rv*) and guanylate cyclase-activating proteins (*GCAPs*). *Rv* binds and inhibits G-protein-coupled receptor kinase 1 (*GRK*1). *GCAPs* activate de novo synthesis of *cGMP* by guanylate cyclases (*GC*). During the recovery phase, *GRK*1 phosphorylates *R** to enable its quenching by arrestin (*Arr*1). The intrinsic GTPase activity of transducin α subunit hydrolyzes *GTP* to *GDP*. The GTPase activity is accelerated by a GTPase activating protein (*GAP*) complex that consists of *RGS9–1, Gβ5L,* and RGS9 anchoring protein (*R9AP*)

ease of obtaining sufficient outer segments enabled a detailed biochemical characterization of reactions involved in phototransduction activation and deactivation, with many enzymatic activities unambiguously assigned to homogeneously purified outer segment proteins [29, 31, 42, 43, 57, 103]. Back when recombinant DNA was at its fledgling stage, this strong foundation together with electrophysiological interrogations of the system jointly infused the G protein field with enviable real-time kinetic information that still remains unparalleled today [9]. By virtue of its simplicity and accessibility, rod phototransduction has been studied extensively and regarded as one of the most exquisite examples of G-protein signaling pathways. In the dark, a "dark current" circulates between the outer and inner segments of rods and sustains a depolarized membrane potential and continuous glutamate release at axonal terminals. As depicted in Fig. 2.1, illumination activates a G-protein-coupled visual pigment (rhodopsin) located on disc membranes when the attached chromophore 11-*cis*-retinal is photoisomerized into the all-*trans* configuration. The

protein then adopts a conformation capable of catalyzing the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the α-subunit of the G protein heterotrimer named transducin. During its lifetime, a photoactivated rhodopsin molecule may activate ~20 transducin in a mouse rod [54]. The GTP-bound transducin α-subunit dissociates from the βγ dimer to interact with the effector cGMP phosphodiesterase (PDE) by sequestering its inhibitory γ-subunit (PDEγ). The disinhibited PDE catalytic αβ subunits are near perfect enzymes that hydrolyze cGMP at a rate near the diffusion limit [4, 59]. This leads to a hasty decline in free cGMP concentration and closure of cyclic-nucleotide-gated (CNG) channels, which otherwise allow extracellular Na^+, Ca^{2+}, and Mg^{2+} [16, 109] to enter the outer segment to drive the dark current. The interruption of inward current causes membrane hyperpolarization and a reduction in tonic synaptic glutamate release. The activities of rhodopsin and PDE endow the pathway with the needed amplification and together with the remarkable thermal stability of rhodopsin in the dark [111], they account for the long-appreciated fact that a human rod can detect single photons [38]. In contrast to the light-induced decrease of Ca^{2+} influx through CNG channels, the light-insensitive and electrogenic Na^+/Ca^{2+}, K^+ exchanger (NCKX) located on the outer segment plasma membrane extrudes Ca^{2+} continuously during channel closure [110]. This leads to a drop in intracellular Ca^{2+} level, measured in both rods and cones of different species to change from 600 to 700 nM in the dark to <50 nM in saturating light [33, 83, 84, 104]. The decline of cytoplasmic Ca^{2+} concentration regulates several reactions involved in phototransduction inactivation.

The overall duration of a rod photoresponse, and the temporal sensitivity of rod vision, is determined by how fast phototransduction is terminated. For deactivation to occur in a timely manner, all active intermediates need to be turned off and cGMP levels restored. At the receptor level, the activated rhodopsin is phosphorylated by rhodopsin kinases (aka G-protein-coupled receptor kinase 1 (GRK1)) and then bound by arrestin to cap its catalytic ability. Rhodopsin kinase activity is regulated by Ca^{2+} through its Ca^{2+}-dependent interaction with recoverin, the study of which exemplifies the power of parallel characterizations of phototransduction using biochemical and electrophysiological means. As a small photoreceptor-enriched calcium-binding protein, recoverin was initially thought to stimulate guanylate cyclase (GC) activity [23]; but see also [45] when intracellular calcium levels fall following light stimulation—this allows the rapid restoration of cGMP needed for response recovery [50]. However, when internally dialyzed into outer segments, exogenous recoverin did not alter recovery kinetics but instead prolonged the activation phase [26, 34], quite the opposite of what one might have expected for a protein involved in recovery. These results necessitated another search for new calcium-binding protein(s) for this particular activity, leading to the identification of guanylate cyclase-activating proteins (GCAPs) [24, 32]. The GCAP-activated and GC-dependent de novo cGMP synthesis replenishes free cGMP levels in the outer segment, facilitating the reopening of CNG channels and depolarization of rods. As for recoverin, it binds and inhibits rhodopsin kinase [10, 46, 49], thus participating in recovery in a counterintuitive way [19, 66]. Increasing recoverin levels in the outer segment prolongs phototransduction by holding back rhodopsin kinase to

prevent phosphorylation of activated rhodopsin, and hence delays its quenching mediated by subsequent arrestin binding. At the G protein level, the α-subunit itself has intrinsic GTPase activity, which can be accelerated by the GTPase-activating protein (GAP) complex comprising RGS9-1, Gβ5-L, and R9AP. The GAP activity can be further enhanced by PDEγ, ensuring timely deactivation of transducin only after its interaction with PDE. Details regarding the discovery of the transducin GAP complex will be discussed in more detail below.

Akin to rods, cone phototransduction uses cGMP as the second messenger but signaling is carried out by a distinct set of proteins. For example, instead of rhodopsin, cones express visual pigments of distinctive spectral sensitivities, or opsins, to enable color vision. Furthermore, some proteins such as RGS9-1 and rhodopsin kinase are expressed by both rods and cones albeit to different levels, which may also contribute to the dissimilar response kinetics and light/dark adaptation properties between the two cell types.

The GAP for Transducin Is a Protein Complex

Another windfall from both biochemical and physiological characterizations of phototransduction is the insight that transducin required a GAP for its timely deactivation [2, 3]. Depending on species and recording conditions, a typical photoresponse of a vertebrate rod lasts for a few hundred milliseconds [9]. In order for response recovery to occur, all active G proteins must be inactivated, as must all other active intermediates generated during the phototransduction activation phase. In the case of transducin, the deactivation relies on its intrinsic GTPase activity [29, 40], which is assayable by measuring the release of radioactive γ-phosphate of bound GTP. The measured rate of γ-phosphate release from purified transducin was too slow in vitro, roughly once every 10–20 s [2, 3, 40] to commensurate with the short duration of recorded rod photoresponses. Therefore, factor(s) exists in the outer segment that can dramatically accelerate GTP hydrolysis by transducin.

Since the GAP activity is assayable, fractionating outer segment components seemed to be a straightforward way to identify it. However, the GAP activity was found to be tightly associated with membranes and labile in the presence of detergents. Direct purification of the GAP turned out to be a formidable task. However, these attempts were fruitful to establish the role of the inhibitory subunit of the cGMP-PDE [3, 96], which is not the GAP itself but it significantly augments the GAP activity [2, 14]. Around that time, a new group of proteins coined regulators of G-protein signaling (RGS) were discovered in the trimeric G protein field, first in yeast and worm then in mammals [25, 51]. These RGS proteins are quite different in sizes but all possess a highly conserved RGS domain of approximately 120 amino acids in length that is necessary and sufficient for their GAP activity toward $G_{i/o}$ proteins [101]. Several laboratories went on to test the possibility that the long sought-after transducin GAP might be an RGS protein. For several members that were tested, they indeed all helped transducin hydrolyzing bound GTP in vitro [12,

27, 37, 68, 69]. The investigation then turned to determine which RGS protein is the actual transducin GAP, or to accommodate a notion that multiple RGS proteins are redundantly present in the outer segment for this important task. To sort out these possibilities, additional criteria such as photoreceptor-specific expression and whether or not PDEγ could enhance their GAP activity were considered [37, 102]. RGS9-1 stood out as a promising candidate because it satisfied both of these requirements [37], while others did not [102]. The speculation was confirmed as recovery in mouse rod and cone was severely delayed when the RGS9 gene was knocked out [14, 62].

These loss-of-function approaches in genetically malleable species, such as mouse and zebra fish, have proved quite useful to establish the genes critical for phototransduction (for examples, see [13, 58, 63, 71, 80, 106]). In the case of identifying the transducin GAP and in addition to RGS9-1, this approach verified the presence of two more components, namely Gβ5-L [98] and R9AP (RGS9 anchoring protein, [41]), in the ternary GAP complex in photoreceptors that helps transducin hydrolyze GTP. The delays in phototransduction recovery seen in RGS9-knockout rods are also found in rods lacking Gβ5 or R9AP [48]. Furthermore, these studies also revealed a unique obligate partnership between RGS9-1 and Gβ5-L [14, 17, 48], suggested during biochemical characterization of then elusive transducin GAP [65], wherein the loss of one leads to the instability of the other despite the presence of messenger RNA. Equally if not more interesting is that the expression level of this GAP complex is higher in cones than in rods [21]. Depending on the species, this difference can be as high as tenfold [113, 114] and hence fuels the speculation that the GAP level might be an important factor governing kinetic differences between rod and cone photoresponses.

Regulation of Rhodopsin Lifetime

The decay of purified activated rhodopsin in vitro is also too slow to account for the short duration of rod photoresponses. To quickly shut down activated rhodopsin, the C-terminal tail of rhodopsin which contains multiple serine and threonine sites is indispensable [11]. In fact, one of the early biochemical reactions readily characterized in purified outer segment preparations was light-dependent rhodopsin phosphorylation [60, 89]. The kinase responsible for this activity is rhodopsin kinase [44, 61], which belongs to the G protein receptor kinase (GRK) family and is expressed in both rods and cones in humans [15, 55, 115]. Phosphorylation of rhodopsin triggers the binding of arrestin [55], which quenches rhodopsin's catalytic activity and prevents it from activating additional transducin molecules. Human Oguchi disease patients with loss-of-function mutations in either arrestin [28] or rhodopsin kinase gene [108] are night blind because their rods remain saturated due to the prolonged catalytic activity of photoactivated rhodopsin. However, their daytime vision is minimally affected [20, 28, 108] because additional cone arrestin [22] and cone opsin kinase (aka GRK7) [15, 99, 100] are present to prevent excessive signaling from

cone visual pigments. When assayed in vitro on rhodopsin, recombinant GRK7 is a superior enzyme to rhodopsin kinase [72, 95]. In carp retina where pure rod and cone populations can be isolated in sufficient quantity for side-by-side comparison, efficiency of light-dependent visual pigment phosphorylation was reported to be much higher in cones than in rods [95]. It is unclear whether the presence of both GRK7 and rhodopsin kinase in cones of certain species, such as humans, contributes to the sensitivity and kinetic differences between the two photoreceptor types. Unlike Oguchi disease patients, however, mice lacking rhodopsin kinase have much delayed cone photoresponse recovery [13, 63]. The lack of GRK7 expression in mouse cones may account for this discord between human and mouse [99]. It is noteworthy that there exist other kinases in photoreceptors that can phosphorylate activated visual pigments. One such kinase that has been extensively characterized is protein kinase C (PKC) [35]. However, in the context of phototransduction recovery, PKC apparently plays little role [13, 63]. Finally, the N-terminal portion of rhodopsin kinase is phosphorylated by protein kinase A (PKA) at serine 21 in dark-adapted retina, which leads to a reduction in catalytic activity toward activated rhodopsin [73]. It was speculated that this regulation is involved in photoreceptor light adaptation, an interesting idea that awaits future investigation.

Rate-Limiting Step of Phototransduction Recovery

The relatively short duration of rod's photoresponses intrigued many laboratories. The activation reactions, initiated by photoisomerization of rhodopsin's chromophore, proceed sequentially in the phototransduction cascade (Fig. 2.1). This activation phase can be robustly recorded from photoreceptors of many species and can be modeled by a delayed Gaussian function put forth by Lamb and Pugh [56]. The LP model can account for the diversity of response parameters due to species difference in outer segment dimensions and different recording temperatures. Unlike the activation phase, however, reactions involved in the recovery phase occur in parallel and all active intermediates produced during activation must be turned off, and cGMP must be replenished to reopen CNG channels to resume the dark current [9]. Conceivably, the slowest reaction(s) among the many that must occur determines the overall rate of recovery. Mechanistic investigations along this line thus aim to identify the so-called rate-limiting step of recovery. Many elegant studies were conducted [47, 54, 67, 79, 81, 112] but thus far the identities of this rate-limiting step appeared mixed, which may reflect species difference or an intrinsic difference between rods and cones within the same species [62, 67, 112]. All experiments, in vitro and in vivo, compared recovery time constants in manipulated versus control conditions, procedurally or reverse-genetically. If the speed of a reaction when accelerated increased the overall rate of recovery, the rate-limiting step was then said to be identified or otherwise excluded. Several seminal findings along this line of thought are worth mentioning. First, it was shown that preventing outer segment calcium changes in salamander rods has no effect on the rate of rod recovery,

indicating that the rate-limiting step therein is a calcium-independent process [62]. However, this finding appears not applicable to cones of the same species as calcium appears to alter the rate of cone recovery when the light-induced drop in outer segment calcium level was delayed [67, 112]. While it is unclear where calcium exerts this effect on cone recovery in salamander, existing data suggest that it is at the pigment level. The identity of the rate-limiting step is a bit clearer in mouse. A loss-of-function approach by inactivating GCAPs has ruled out the involvement of the calcium/cGMP negative-feedback loop [7]. Knocking out the calcium-binding protein recoverin hastens the recovery of rod phototransduction [66]. As mentioned earlier, recoverin binds rhodopsin kinase in a calcium-dependent manner [10, 49], so this deductively implies that rhodopsin deactivation might be the long thought-after rate-limiting step. However, in another gain-of-function experiment, overexpressing rhodopsin kinase by three to four folds either has no significant effect [54] or accelerates rod recovery to a moderate extent [82].

A surprise emerged when elevation of the level of transducin GAP complex by overexpressing its membrane anchor R9AP leads to a dramatic speeding up of rod recovery [54]. The degree of acceleration correlates well with moderate level of overexpression but approaches an asymptote when expression is pushed higher. This is because rod recovery becomes more limited by the next slowest reaction(s) than by transducin deactivation. Taken together, these data indicate pretty clearly that transducin deactivation is the rate-limiting step in normal mouse rod recovery. However, this is a conclusion that needs reconciliation with the observed moderate acceleration of recovery in recoverin-less rods [66], the robust binding and inhibition of rhodopsin kinase by recoverin [10, 49], and moderately accelerated rod recovery when rhodopsin kinase is overexpressed [19, 82]. Given the precedence that calcium appears to differentially regulate amphibian rod and cone phototransduction recovery and that cones express a higher level of transducin GAP than rods [21, 113, 114] in multiple species, it will also be important to see whether transducin deactivation similarly rate-limits mouse cone recovery. Along this line, it is noteworthy that overexpressing GCAP1 was found to speed up the recovery of cone-derived electroretinogram (ERG) responses. However, it remains to be determined whether such acceleration occurs directly at the level of cone phototransduction [76]. Finally, from an evolutionary point of view, it is worthwhile to revisit amphibian phototransduction to determine whether species differences (vs. mouse) account for the disparity in reactions that limit the rate of recovery. For instance, while salamander rod recovery is not influenced by holding outer segment calcium fixed, it is unclear whether G protein deactivation is the rate-limiting step. Similarly, while calcium is important for timely recovery of salamander cones, the identity of the rate-limiting step therein has not been shown by a gain-of-function approach under normal and not under calcium-clamped conditions. The salamander genome has been sequenced and annotated [91] and novel genome-editing tools have emerged [86] that may allow comparative genetic studies in this species with those from laboratory mice. These experiments are necessary to determine the importance of G proteins and their timely deactivation in the recovery of photoreceptor light responses among different species.

The Role of Recoverin in Phototransduction

The binding and inhibition of rhodopsin kinase by recoverin is robust and occurs at physiological calcium levels [8, 10, 49]. The notion that transducin deactivation rate-limits mouse rod recovery needs to be reconciled with the moderately accelerated recovery observed in recoverin-knockout rods [66]. By breeding the recoverin-knockout mice into a background where transducin GAP is highly overexpressed [19, 53], the moderately accelerated rod recovery can be further hastened. This indicates that the rate of recovery in recoverin-knockout rods is rate-limited by transducin deactivation. The moderate acceleration of recovery can also be recorded in mice with greater than tenfold rhodopsin kinase overexpression, which can similarly be speeded further by GAP overexpression [19]. Thus, it appears that in addition to regulating rhodopsin lifetime [18, 26, 34, 44], recoverin and rhodopsin kinase also play an unintuitive role in normal rod recovery by speeding up PDE shutoff, as previously reported [66].

It is unclear how recoverin and rhodopsin kinase regulate PDE deactivation. However, the presence of the GAP complex in mouse rods is required to see this moderate acceleration effect on recovery [19], indicating that transducin, GAP complex, and/or PDE itself may be the target and that the N-terminal RGS homology (RH) domain of rhodopsin kinase [88] cannot substitute as a surrogate transducin GAP. Along these lines, it has been shown that removing phosphorylation sites of the inhibitory γ subunit of PDE alters rod recovery kinetics [97]. Although a direct link between rhodopsin kinase and PDEγ phosphorylation has not been established, it is an interesting target for future investigation. Furthermore, recoverin is abundantly expressed in photoreceptors and the distribution therein is regulated by light [94]. The physiological significance of this light-dependent recoverin redistribution in photoreceptors remains unclear, nor is the mechanism. Despite a lack of mechanistic understanding, the physiological importance of recoverin-mediated acceleration of rod recovery in light adaptation is now appreciated. It is well established that background light accelerates rod recovery [104]. The acceleration is in large part mediated by recoverin's action on rhodopsin kinase because knocking out recoverin hampers it [19]. Thus, with respect to recoverin's role in phototransduction, it appears to modulate the lifetime of activated rhodopsin and to speed up recovery during light adaptation. Finally, one enigmatic function of recoverin remains unsolved as it appears to enhance signal transmission between rod and downstream neurons [85].

Future Directions

Understanding how generalizable the knowledge we gain from phototransduction applies to other trimeric G protein pathways throughout the body is a worthwhile endeavor. Even within the visual system, we still are putting together the tit bits of information available to construct G-protein signaling cascades in retinal depolar-

izing bipolar cells [52, 76, 87] and in intrinsically photosensitive retinal ganglion cells [107]. Future research efforts may benefit from a focused approach in the retina, which is an approachable part of the central nervous system with abundant anatomical detail to assist mechanistic inquisition. While mechanisms of rod phototransduction are well described, cone phototransduction remains incompletely understood. A simultaneous comparative examination of both rods and cones will provide valuable insights into how these mechanisms produce light responses with distinct characteristics. Finally, the field of G-protein signaling in the retina may exert further impact by advancing the biophysical characterizations [78] of important molecules such as visual pigments [74, 93], G proteins [92], G protein GAPs [90], arrestins [39], PDEs [4], rhodopsin kinase [87], GCAPs [1], and GCs, to name just a few, to understand how mutations therein affect complex formation and/or biochemical activity, and how functional perturbations may cause blinding diseases in humans.

References

1. Ames JB, Ikura M (2002) Structure and membrane-targeting mechanism of retinal Ca^{2+}-binding proteins, recoverin and GCAP-2. Adv Exp Med Biol 514:333–348
2. Angleson JK, Wensel TG (1993) A GTPase-accelerating factor for transducin, distinct from its effector cGMP phosphodiesterase, in rod outer segment membranes. Neuron 11(5):939–949
3. Arshavsky V, Bownds MD (1992) Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. Nature 357(6377):416–417
4. Baehr W, Frederick JM (2009) Naturally occurring animal models with outer retina phenotypes. Vision Res 49(22):2636–2252
5. Baehr W, Devlin MJ, Applebury ML (1979) Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments. J Biol Chem 254(22):11669–11677
6. Barren B, Gakhar L, Muradov H, Boyd KK, Ramaswamy S, Artemyev NO (2009) Structural basis of phosphodiesterase 6 inhibition by the C-terminal region of the gamma-subunit. EMBO J 28(22):3613–3622
7. Burns ME, Mendez A, Chen J, Baylor DA (2002) Dynamics of cyclic GMP synthesis in retinal rods. Neuron 36(1):81–91
8. Chen CK (2002) Recoverin and rhodopsin kinase. Adv Exp Med Biol 514:101–107
9. Chen CK (2005) The vertebrate phototransduction cascade: amplification and termination mechanisms. Rev Physiol Biochem Pharmacol 154:101–121
10. Chen CK, Inglese J, Lefkowitz RJ, Hurley JB (1995a) Ca(2+)-dependent interaction of recoverin with rhodopsin kinase. J Biol Chem 270(30):18060–18066
11. Chen J, Makino CL, Peachey NS, Baylor DA, Simon MI (1995b) Mechanisms of rhodopsin inactivation in vivo as revealed by a COOH-terminal truncation mutant. Science 267(5196):374–377
12. Chen CK, Wieland T, Simon MI (1996) RGS-r, a retinal specific RGS protein, binds an intermediate conformation of transducin and enhances recycling. Proc Natl Acad Sci U S A 93(23):12885–12889
13. Chen CK, Burns ME, Spencer M, Niemi GA, Chen J, Hurley JB et al (1999) Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. Proc Natl Acad Sci U S A 96(7):3718–3722

14. Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI (2000) Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. Nature 403(6769):557–560
15. Chen CK, Zhang K, Church-Kopish J, Huang W, Zhang H, Chen YJ et al (2001) Characterization of human GRK7 as a potential cone opsin kinase. Mol Vis 7:305–313
16. Chen C, Nakatani K, Koutalos Y (2003a) Free magnesium concentration in salamander photoreceptor outer segments. J Physiol 553(Pt 1):125–135
17. Chen CK, Eversole-Cire P, Zhang H, Mancino V, Chen YJ, He W et al (2003b) Instability of GGL domain-containing RGS proteins in mice lacking the G protein beta-subunit Gbeta5. Proc Natl Acad Sci U S A 100(11):6604–6609
18. Chen CK, Woodruff ML, Chen FS, Chen D, Fain GL (2010) Background light produces a recoverin-dependent modulation of activated-rhodopsin lifetime in mouse rods. J Neurosci 30(4):1213–1220
19. Chen CK, Woodruff ML, Chen FS, Chen Y, Cilluffo MC, Tranchina D et al (2012) Modulation of mouse rod response decay by rhodopsin kinase and recoverin. J Neurosci 32(45):15998–16006
20. Cideciyan AV, Zhao X, Nielsen L, Khani SC, Jacobson SG, Palczewski K (1998) Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man. Proc Natl Acad Sci U S A 95(1):328–333
21. Cowan CW, Fariss RN, Sokal I, Palczewski K, Wensel TG (1998) High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. Proc Natl Acad Sci U S A 95(9):5351–5356
22. Craft CM, Whitmore DH, Wiechmann AF (1994) Cone arrestin identified by targeting expression of a functional family. J Biol Chem 269(6):4613–4619
23. Dizhoor AM, Ray S, Kumar S, Niemi G, Spencer M, Brolley D et al (1991) Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. Science 251(4996):915–918
24. Dizhoor AM, Olshevskaya EV, Henzel WJ, Wong SC, Stults JT, Ankoudinova I et al (1995) Cloning, sequencing, and expression of a 24-kDa Ca(2+)-binding protein activating photoreceptor guanylyl cyclase. J Biol Chem 270(42):25200–25206
25. Dohlman HG, Apaniesk D, Chen Y, Song J, Nusskern D (1995) Inhibition of G-protein signaling by dominant gain-of-function mutations in Sst2p, a pheromone desensitization factor in Saccharomyces cerevisiae. Mol Cell Biol 15(7):3635–3643
26. Erickson MA, Lagnado L, Zozulya S, Neubert TA, Stryer L, Baylor DA (1998) The effect of recombinant recoverin on the photoresponse of truncated rod photoreceptors. Proc Natl Acad Sci U S A 95(11):6474–6479
27. Faurobert E, Hurley JB (1997) The core domain of a new retina specific RGS protein stimulates the GTPase activity of transducin in vitro. Proc Natl Acad Sci U S A 94(7):2945–2950
28. Fuchs S, Nakazawa M, Maw M, Tamai M, Oguchi Y, Gal A (1995) A homozygous 1-base pair deletion in the arrestin gene is a frequent cause of Oguchi disease in Japanese. Nat Genet 10(3):360–362
29. Fung BK (1983) Characterization of transducin from bovine retinal rod outer segments. I. Separation and reconstitution of the subunits. J Biol Chem 258(17):10495–10502
30. Fung BK, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc Natl Acad Sci U S A 78(1):152–156
31. Fung BK, Lieberman BS, Lee RH (1992) A third form of the G protein beta subunit. 2. Purification and biochemical properties. J Biol Chem 267(34):24782–24788
32. Gorczyca WA, Polans AS, Surgucheva IG, Subbaraya I, Baehr W, Palczewski K (1995) Guanylyl cyclase activating protein. A calcium-sensitive regulator of phototransduction. J Biol Chem 270(37):22029–22036
33. Gray-Keller MP, Detwiler PB (1994) The calcium feedback signal in the phototransduction cascade of vertebrate rods. Neuron 13(4):849–861
34. Gray-Keller MP, Polans AS, Palczewski K, Detwiler PB (1993) The effect of recoverin-like calcium-binding proteins on the photoresponse of retinal rods. Neuron 10(3):523–531

35. Greene NM, Williams DS, Newton AC (1995) Kinetics and localization of the phosphorylation of rhodopsin by protein kinase C. J Biol Chem 270(12):6710–6717
36. Greene NM, Williams DS, Newton AC (1997) Identification of protein kinase C phosphorylation sites on bovine rhodopsin. J Biol Chem 272(16):10341–10344
37. He W, Cowan CW, Wensel TG (1998) RGS9, a GTPase accelerator for phototransduction. Neuron 20(1):95–102
38. Hecht S, Shlaer S, Pirenne MH (1941) Energy at the threshold of vision. Science 93(2425):585–587
39. Hirsch JA, Schubert C, Gurevich VV, Sigler PB (1999) A model for Arrestin's regulation: the 2.8 Å crystal structure of visual Arrestin. Cell 97(2):257–269
40. Ho YK, Hingorani VN, Navon SE, Fung BK (1989) Transducin: a signaling switch regulated by guanine nucleotides. Curr Top Cell Regul 30:171–202
41. Hu G, Wensel TG (2002) R9AP, a membrane anchor for the photoreceptor GTPase accelerating protein, RGS9-1. Proc Natl Acad Sci U S A 99(15):9755–9760
42. Hurley JB, Stryer L (1982) Purification and characterization of the gamma regulatory subunit of the cyclic GMP phosphodiesterase from retinal rod outer segments. J Biol Chem 257(18):11094–11099
43. Hurwitz RL, Bunt-Milam AH, Chang ML, Beavo JA (1985) cGMP phosphodiesterase in rod and cone outer segments of the retina. J Biol Chem 260(1):568–573
44. Inglese J, Koch WJ, Caron MG, Lefkowitz RJ (1992) Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinases. Nature 359(6391):147–150
45. Kawamura S (1992) Light-sensitivity modulating protein in frog rods. Photochem Photobiol 56(6):1173–1180
46. Kawamura S (1993) Rhodopsin phosphorylation as a mechanism of cyclic GMP phosphodiesterase regulation by S-modulin. Nature 362(6423):855–857
47. Kennedy MJ, Sowa ME, Wensel TG, Hurley JB (2003) Acceleration of key reactions as a strategy to elucidate the rate-limiting chemistry underlying phototransduction inactivation. Invest Ophthalmol Vis Sci 44(3):1016–1022
48. Keresztes G, Martemyanov KA, Krispel CM, Mutai H, Yoo PJ, Maison SF et al (2004) Absence of the RGS9.Gbeta5 GTPase-activating complex in photoreceptors of the R9AP knockout mouse. J Biol Chem 279(3):1581–1584
49. Klenchin VA, Calvert PD, Bownds MD (1995) Inhibition of rhodopsin kinase by recoverin. Further evidence for a negative feedback system in phototransduction. J Biol Chem 270(27):16147–16152
50. Koch KW, Stryer L (1988) Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. Nature 334(6177):64–66
51. Koelle MR, Horvitz HR (1996) EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins. Cell 84(1):115–125
52. Koike C, Obara T, Uriu Y, Numata T, Sanuki R, Miyata K et al (2010) TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. Proc Natl Acad Sci U S A 107(1):332–337
53. Krispel CM, Chen CK, Simon MI, Burns ME (2003) Prolonged photoresponses and defective adaptation in rods of Gbeta5-/- mice. J Neurosci 23(18):6965–6971
54. Krispel CM, Chen D, Melling N, Chen YJ, Martemyanov KA, Quillinan N et al (2006) RGS expression rate-limits recovery of rod photoresponses. Neuron 51(4):409–416
55. Kuhn H, Wilden U (1987) Deactivation of photoactivated rhodopsin by rhodopsin-kinase and arrestin. J Recept Res 7(1–4):283–298
56. Lamb TD, Pugh EN Jr (1992) A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. J Physiol 449:719–758
57. Lee RH, Lieberman BS, Lolley RN (1987) A novel complex from bovine visual cells of a 33,000-dalton phosphoprotein with beta- and gamma-transducin: purification and subunit structure. BioChemistry 26(13):3983–3990

58. Lem J, Makino CL (1996) Phototransduction in transgenic mice. Curr Opin Neurobiol 6(4):453–458
59. Leskov IB, Klenchin VA, Handy JW, Whitlock GG, Govardovskii VI, Bownds MD et al (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. Neuron 27(3):525–537
60. Liebman PA, Pugh EN (1980) Jr. ATP mediates rapid reversal of cyclic GMP phosphodiesterase activation in visual receptor membranes. Nature 287(5784):734–736
61. Lorenz W, Inglese J, Palczewski K, Onorato JJ, Caron MG, Lefkowitz RJ (1991) The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the beta-adrenergic receptor kinase. Proc Natl Acad Sci U S A 88(19):8715–8719
62. Lyubarsky A, Nikonov S, Pugh EN Jr (1996) The kinetics of inactivation of the rod phototransduction cascade with constant Ca2+i. J Gen Physiol 107(1):19–34
63. Lyubarsky AL, Chen C, Simon MI, Pugh EN Jr (2000) Mice lacking G-protein receptor kinase 1 have profoundly slowed recovery of cone-driven retinal responses. J Neurosci 20(6):2209–2217
64. Lyubarsky AL, Naarendorp F, Zhang X, Wensel T, Simon MI, Pugh EN Jr (2001) RGS9-1 is required for normal inactivation of mouse cone phototransduction. Mol Vis 7:71–78
65. Makino ER, Handy JW, Li T, Arshavsky VY (1999) The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein beta subunit. Proc Natl Acad Sci U S A 96(5):1947–1952
66. Makino CL, Dodd RL, Chen J, Burns ME, Roca A, Simon MI et al (2004) Recoverin regulates light-dependent phosphodiesterase activity in retinal rods. J Gen Physiol 123(6):729–741
67. Matthews HR, Sampath AP (2010) Photopigment quenching is Ca^{2+} dependent and controls response duration in salamander L-cone photoreceptors. J Gen Physiol 135(4):355–366
68. Morrow EM, Furukawa T, Cepko CL (1998) Vertebrate photoreceptor cell development and disease. Trends Cell Biol 8(9):353–358
69. Natochin M, Granovsky AE, Artemyev NO (1997) Regulation of transducin GTPase activity by human retinal RGS. J Biol Chem 272(28):17444–17449
70. Nekrasova ER, Berman DM, Rustandi RR, Hamm HE, Gilman AG, Arshavsky VY (1997) Activation of transducin guanosine triphosphatase by two proteins of the RGS family. Bio-Chemistry 36(25):7638–7643
71. Nikonov SS, Brown BM, Davis JA, Zuniga FI, Bragin A, Pugh EN Jr (2008) et al. Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 59(3):462–474
72. Osawa S, Jo R, Weiss ER (2008) Phosphorylation of GRK7 by PKA in cone photoreceptor cells is regulated by light. J Neurochem 107(5):1314–1324
73. Osawa S, Jo R, Xiong Y, Reidel B, Tserentsoodol N, Arshavsky VY et al (2011) Phosphorylation of G protein-coupled receptor kinase 1 (GRK1) is regulated by light but independent of phototransduction in rod photoreceptors. J Biol Chem 286(23):20923–20929
74. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA et al (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289(5480):739–745
75. Papermaster DS (1982) Preparation of retinal rod outer segments. Methods Enzymol 81:48–52
76. Pearring JN, Bojang P Jr, Shen Y, Koike C, Furukawa T, Nawy S et al (2011) A role for nyctalopin, a small leucine-rich repeat protein, in localizing the TRP melastatin 1 channel to retinal depolarizing bipolar cell dendrites. J Neurosci 31(27):10060–10066
77. Pennesi ME, Howes KA, Baehr W, Wu SM (2003) Guanylate cyclase-activating protein (GCAP) 1 rescues cone recovery kinetics in GCAP1/GCAP2 knockout mice. Proc Natl Acad Sci U S A 100(11):6783–6788
78. Ridge KD, Abdulaev NG, Sousa M, Palczewski K (2003) Phototransduction: crystal clear. Trends Biochem Sci 28(9):479–487
79. Rieke F, Baylor DA (1998) Origin of reproducibility in the responses of retinal rods to single photons. Biophys J 75(4):1836–1857

80. Rinner O, Makhankov YV, Biehlmaier O, Neuhauss SC (2005) Knockdown of cone-specific kinase GRK7 in larval zebrafish leads to impaired cone response recovery and delayed dark adaptation. Neuron 47(2):231–242
81. Sagoo MS, Lagnado L (1997) G-protein deactivation is rate-limiting for shut-off of the phototransduction cascade. Nature 389(6649):392–395
82. Sakurai K, Young JE, Kefalov VJ, Khani SC (2011) Variation in rhodopsin kinase expression alters the dim flash response shut off and the light adaptation in rod photoreceptors. Invest Ophthalmol Vis Sci 52(9):6793–6800
83. Sampath AP, Matthews HR, Cornwall MC, Fain GL (1998) Bleached pigment produces a maintained decrease in outer segment Ca^{2+} in salamander rods. J Gen Physiol 111(1):53–64
84. Sampath AP, Matthews HR, Cornwall MC, Bandarchi J, Fain GL (1999) Light-dependent changes in outer segment free-Ca2+concentration in salamander cone photoreceptors. J Gen Physiol 113(2):267–277
85. Sampath AP, Strissel KJ, Elias R, Arshavsky VY, McGinnis JF, Chen J et al (2005) Recoverin improves rod-mediated vision by enhancing signal transmission in the mouse retina. Neuron 46(3):413–420
86. Sampson TR, Weiss DS (2014) Exploiting CRISPR/Cas systems for biotechnology. Bioessays 36(1):34–38
87. Shen Y, Rampino MA, Carroll RC, Nawy S (2012) G-protein-mediated inhibition of the Trp channel TRPM1 requires the Gbetagamma dimer. Proc Natl Acad Sci U S A 109(22):8752–8757
88. Singh P, Wang B, Maeda T, Palczewski K, Tesmer JJ (2008) Structures of rhodopsin kinase in different ligand states reveal key elements involved in G protein-coupled receptor kinase activation. J Biol Chem 283(20):14053–14062
89. Sitaramayya A, Liebman PA (1983) Phosphorylation of rhodopsin and quenching of cyclic GMP phosphodiesterase activation by ATP at weak bleaches. J Biol Chem 258(20):12106–12109
90. Slep KC, Kercher MA, He W, Cowan CW, Wensel TG, Sigler PB (2001) Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 Å. Nature 409(6823):1071–1077
91. Smith JJ, Putta S, Walker JA, Kump DK, Samuels AK, Monaghan JR et al (2005) Sal-site: integrating new and existing ambystomatid salamander research and informational resources. BMC Genomics 6:181
92. Sondek J, Lambright DG, Noel JP, Hamm HE, Sigler PB (1994) GTPase mechanism of Gproteins from the 1.7-A crystal structure of transducin alpha-GDP-AIF-4. Nature 372(6503):276–279
93. Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, Oprian DD et al (2011) The structural basis of agonist-induced activation in constitutively active rhodopsin. Nature 471(7340):656–660
94. Strissel KJ, Lishko PV, Trieu LH, Kennedy MJ, Hurley JB, Arshavsky VY (2005) Recoverin undergoes light-dependent intracellular translocation in rod photoreceptors. J Biol Chem 280(32):29250–29255
95. Tachibanaki S, Arinobu D, Shimauchi-Matsukawa Y, Tsushima S, Kawamura S (2005) Highly effective phosphorylation by G protein-coupled receptor kinase 7 of light-activated visual pigment in cones. Proc Natl Acad Sci U S A 102(26):9329–9334
96. Tsang SH, Burns ME, Calvert PD, Gouras P, Baylor DA, Goff SP (1998) et al. Role for the target enzyme in deactivation of photoreceptor G protein in vivo. Science 282(5386):117–121
97. Tsang SH, Woodruff ML, Janisch KM, Cilluffo MC, Farber DB, Fain GL (2007) Removal of phosphorylation sites of gamma subunit of phosphodiesterase 6 alters rod light response. J Physiol 579(Pt 2):303–312
98. Watson AJ, Aragay AM, Slepak VZ, Simon MI (1996) A novel form of the G protein beta subunit Gbeta5 is specifically expressed in the vertebrate retina. J Biol Chem 271(45):28154–28160

99. Weiss ER, Ducceschi MH, Horner TJ, Li A, Craft CM, Osawa S (2001) Species-specific differences in expression of G-protein-coupled receptor kinase (GRK) 7 and GRK1 in mammalian cone photoreceptor cells: implications for cone cell phototransduction. J Neurosci 21(23):9175–9184
100. Weiss ER, Raman D, Shirakawa S, Ducceschi MH, Bertram PT, Wong F et al (1998) The cloning of GRK7, a candidate cone opsin kinase, from cone- and rod-dominant mammalian retinas. Mol Vis 4:27
101. Wieland T, Chen CK (1999) Regulators of G-protein signalling: a novel protein family involved in timely deactivation and desensitization of signalling via heterotrimeric G proteins. Naunyn Schmiedebergs Arch Pharmacol 360(1):14–26
102. Wieland T, Chen CK, Simon MI (1997) The retinal specific protein RGS-r competes with the gamma subunit of cGMP phosphodiesterase for the alpha subunit of transducin and facilitates signal termination. J Biol Chem 272(14):8853–8856
103. Wilden U, Wust E, Weyand I, Kuhn H (1986) Rapid affinity purification of retinal arrestin (48 kDa protein) via its light-dependent binding to phosphorylated rhodopsin. FEBS Lett 207(2):292–295
104. Woodruff ML, Sampath AP, Matthews HR, Krasnoperova NV, Lem J, Fain GL (2002) Measurement of cytoplasmic calcium concentration in the rods of wild-type and transducin knock-out mice. J Physiol 542(Pt 3):843–854
105. Woodruff ML, Janisch KM, Peshenko IV, Dizhoor AM, Tsang SH, Fain GL (2008) Modulation of phosphodiesterase6 turnoff during background illumination in mouse rod photoreceptors. J Neurosci 28(9):2064–2074
106. Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J (1997) Prolonged photoresponses in transgenic mouse rods lacking arrestin. Nature 389(6650):505–509
107. Xue T, Do MT, Riccio A, Jiang Z, Hsieh J, Wang HC et al (2011) Melanopsin signalling in mammalian iris and retina. Nature 479(7371):67–73
108. Yamamoto S, Sippel KC, Berson EL, Dryja TP (1997) Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness. Nat Genet 15(2):175–178
109. Yau KW (1994) Phototransduction mechanism in retinal rods and cones. The Friedenwald lecture. Invest Ophthalmol Vis Sci 35(1):9–32
110. Yau KW, Nakatani K (1984) Cation selectivity of light-sensitive conductance in retinal rods. Nature 309(5966):352–354
111. Yau KW, Matthews G, Baylor DA (1979) Thermal activation of the visual transduction mechanism in retinal rods. Nature 279(5716):806–807
112. Zang J, Matthews HR (2012) Origin and control of the dominant time constant of salamander cone photoreceptors. J Gen Physiol 140(2):219–233
113. Zhang K, Howes KA, He W, Bronson JD, Pettenati MJ, Chen C et al (1999) Structure, alternative splicing, and expression of the human RGS9 gene. Gene 240(1):23–34
114. Zhang X, Wensel TG, Kraft TW (2003) GTPase regulators and photoresponses in cones of the eastern chipmunk. J Neurosci 23(4):1287–1297
115. Zhao X, Huang J, Khani SC, Palczewski K (1998) Molecular forms of human rhodopsin kinase (GRK1). J Biol Chem 273(9):5124–5131

Chapter 3
Signaling by Rod and Cone Photoreceptors: Opsin Properties, G-protein Assembly, and Mechanisms of Activation

Alexander V. Kolesnikov, Oleg G. Kisselev and Vladimir J. Kefalov

Abstract Photoreceptor cells utilize a G-protein-mediated signaling cascade to convert the energy of a photon into an electric impulse in the first step of vision. The relative abundance of photoreceptors in the retina and the localization of the phototransduction cascade proteins in a specialized modified cilium structure, known as the outer segment (OS), have greatly facilitated the biochemical characterization of phototransduction proteins and the reactions involved in this canonical G protein cascade. In addition, the electrophysiologically measurable response produced by stimulating the cascade with light has resulted in quantitative understanding of the reactions involved in the activation and inactivation of rod and cone photoreceptor G-protein-coupled receptors (GPCRs) and their coupled G protein, transducin. Here, we review the structure of rod and cone visual pigments in the context of photoreceptor function as well as the assembly and function of the heterotrimeric transducin.

Retinal Photoreceptors and Visual Pigments

We derive most of our information about the world through our visual system by means of rod and cone photoreceptors. The human retina has one type of rod for dim-light vision, and three types of cone cells that allow color discrimination. Rods and cones share the same principles of phototransduction, the intracellular mechanism of

A. V. Kolesnikov (✉) · V. J. Kefalov
Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, 660 S. Euclid Ave, McMillan Hospital, room 625, St. Louis, MO 63110, USA
e-mail: kolesnikov@wustl.edu

V. J. Kefalov
e-mail: kefalov@wustl.edu

O. G. Kisselev
Departments of Ophthalmology and Biochemistry and Molecular Biology, Saint Louis University School of Medicine, 1402 S. Grand Blvd., Saint Louis, MO 63104, USA
e-mail: kisselev@slu.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,* Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_3

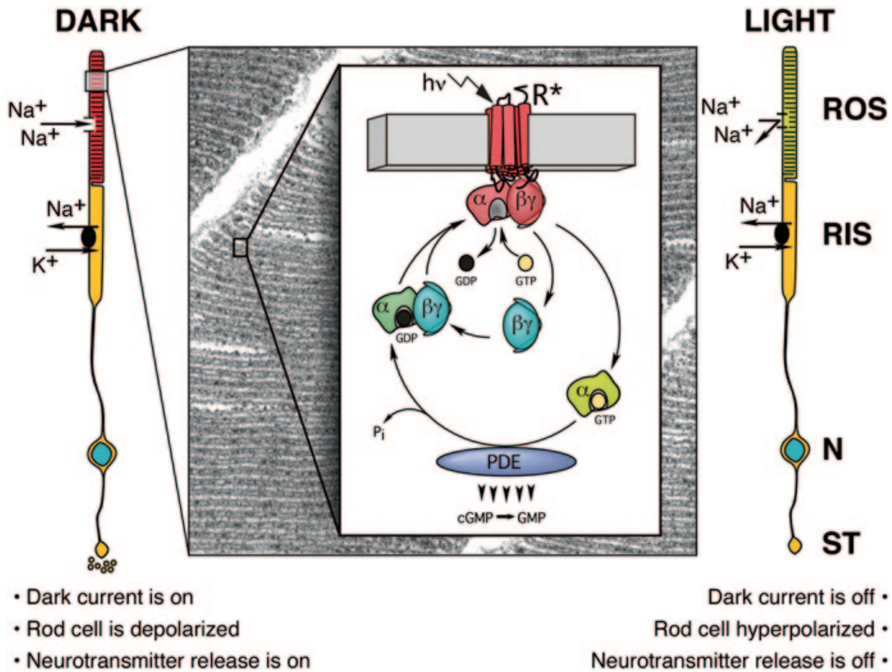

Fig. 3.1 G-protein-mediated signaling in retinal rod photoreceptors. *ROS* rod outer segment, *RIS* rod inner segment, *N* nucleus, *ST* synaptic terminal, *R** photoactivated rhodopsin, α and βγ transducin subunits, *PDE* phosphodiesterase, *GTP* guanosine triphosphate, *GDP* guanosine diphosphate, *cGMP* cyclic guanosine monophosphate, *GMP* guanosine monophosphate, *Pi* inorganic phosphate

light detection. Electrophysiologically, rod and cone photoresponses can be observed either as reduction in the membrane current using single-cell suction electrode recordings or as change in transmembrane potential using electroretinogram (ERG) recordings in vivo or from isolated retina. The pathway for converting light into an electric impulse, known as phototransduction (Fig. 3.1), is a prototypical G-protein-coupled receptor (GPCR) transduction cascade and has been well characterized in rods (for reviews, see [1–3]). There, phototransduction is initiated when a photon absorbed by the visual pigment induces 11-*cis* to all-*trans* conformational change of its chromophore, retinal. The photoactivated visual pigment triggers the next step in the phototransduction cascade by binding to the G protein transducin. This causes the exchange of guanosine diphosphate (GDP) with guanosine-5′-triphosphate (GTP) on the α-subunit of transducin ($G\alpha_t$) converting it to its active form. The $G\alpha_t$–GTP complex, in turn, activates the enzyme cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE), which hydrolyzes cGMP and causes a rapid decrease in its concentration. As a result, cGMP-gated cationic channels in the plasma membrane close, which leads to reduction in membrane current, hyperpolarization of the cell, and modulation of neurotransmitter release at its axon terminal (Fig. 3.1). A similar cascade is believed to function in cones and, indeed, most of the equivalent cone transduction proteins have been identified [4]. Yet, amazingly, the physiological properties of rods and cones

differ significantly in several important aspects that result in two types of photoreceptors suitable for distinct dim- and bright-light conditions, respectively.

The light-absorbing molecule responsible for the initiation of the light response is known as visual pigment. It represents approximately 95 % of the membrane protein in the outer segments of photoreceptor [5]. The visual pigment in both rods and cones consists of an apoprotein, called opsin, and of a chromophore, typically 11-*cis*-retinal [6], but in some aquatic species 11-*cis*-3-dehydroretinal [7]. Retinal is tightly bound in the highly hydrophobic core of the protein [8] and is covalently attached to it [9, 10] via protonated Schiff-base linkage between the aldehyde of retinal and the ε-amino group of a lysine residue [11]. The site of retinal attachment to opsin has been identified, and in vertebrate rod opsin it is Lys-296 (reviewed by [12]). Vertebrate cone opsins also have a lysine at the site corresponding to position 296 of the rod opsin [5].

One productive approach to understanding the interactions between 11-*cis*-retinal and opsin has been to use modified retinal analogs. By modifying the structure of the chromophore and then investigating the characteristics of the resulting pigment, much has been learned about the interaction between retinal and opsin and the relation between their structure and function (for reviews, see [13–16]). The inability of some retinal analogs to form pigment has also provided valuable information about the chromophore-binding pocket of opsin and the constraints it imposes on retinal. The use of smaller compounds has allowed a more precise definition of the structural requirements of the hydrophobic binding of retinal to opsin.

In addition to understanding the basic principles of ligand–opsin interactions, a new aspect of this research has emerged recently that caries significant potential for therapeutic application to treat some of the most debilitating eye diseases that affect photopigment regeneration, such as Leber's congenital amaurosis (LCA), reviewed in [17]. LCA is an inherited eye disease that is characterized by fast onset and rapid retinal degeneration that leads to blindness if left untreated. Although some forms have been successfully cured by gene therapy [18], more treatment options are clearly needed. One of the promising approaches currently under investigation is based on synthetic ligands that bind to the retinal photopigments. Binding of the ligand appears to have beneficial effects via, at least, two mechanisms: (1) stabilization of the opsin conformation and proper delivery to the outer segments [19], and (2) inhibition of the constitutive activity of unliganded opsin by 11-*cis*-retinal or its light-stable analogs [20]. The work on the opsin–retinal interactions represents one of the success stories that align modern physiology, biochemistry, and high-resolution structural studies for the ultimate benefit of patients.

Differences in the Functional Characteristics of Rods and Cones

Despite the similarity in the components of the rod and cone phototransduction cascades, rods and cones differ in a number of ways: rod responses are slower, rods are much more sensitive, and rod photoactivated pigment decays and regenerates

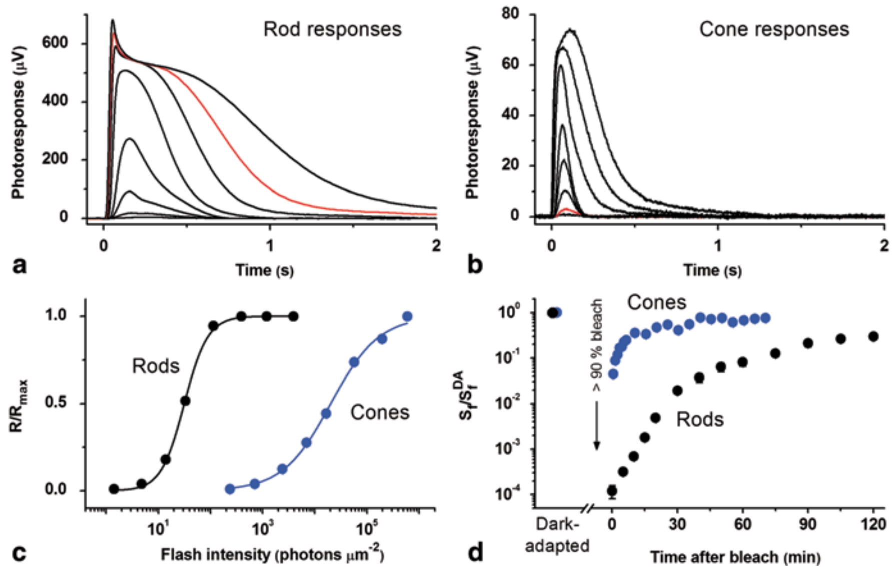

Fig. 3.2 Physiological features of mouse rods and cones. **a** Family of transretinal ERG rod responses to increasing light intensities (wild type mouse). **b** Family of transretinal ERG cone responses to increasing light intensities (*Gnat1*$^{-/-}$ mouse). The *red traces* in **a** and **b** indicate responses to the same light intensity. **c** Normalized intensity–response functions demonstrating ~2.5 log units' lower sensitivity of cones as compared to rods. **d** Faster recovery of fractional sensitivity (S_f) of cones (ERG b-wave) as compared to rods (ERG a-wave) following >90% pigment bleach in vivo. *DA* dark-adapted conditions

much more slowly than cone pigment. The major physiological differences in the two types of photoreceptors are discussed in detail below.

Rod and cone photoreceptors have different but overlapping ranges of operation (Fig. 3.2). Rods are extremely sensitive and capable of detecting even the absorption of a single photon [21, 22]. Thus, the rod cell has acquired the maximum theoretical sensitivity, limited by the quantal nature of light. The reliability of the system and the chance of interference between thermal noise and signal are greatly reduced by the requirement of 6 coincidently stimulated rods for the perception of light ([22]; see also [23]). Rods decrease their sensitivity when exposed to steady illumination and this allows them to extend their functional range over a 6-log unit range of light intensity [24, 25]. However, at light intensity close to twilight conditions, rods saturate and become unresponsive [26, 27]. During the daylight hours, rods are largely saturated and the visual system relies mostly on the cones [28]. Cones are far less sensitive than rods and they require between 100 and 1000 times brighter light to produce a threshold response [25, 29–31]. However, they are able to adapt to much brighter lighting conditions and, in fact, cannot be saturated even with very bright illumination [25, 32, 33]. Thus, rods with their very high sensitivity are perfectly adapted to work in very dim surrounding light and are responsible for nighttime vision. Cones on the other hand function in much brighter conditions

and are responsible for daylight vision. Together, rods and cones allow the human visual system to respond to light over a range of 10^{12}-fold out of the 10^{15}-fold range of intensities normally encountered in nature [26].

An interesting exception to the rule that rods saturate in high light intensity conditions are the rods of the skate which continue to give incremental responses even in extremely bright backgrounds without saturating [34]. This allows the all-rod retina of the skate to remain functional over a wide range of light intensities, from darkness to bright background [35]. The mechanisms of adaptation of skate photoreceptor are not understood. Skate rods, with their physiology resembling that of rods at low light intensities and cones at higher backgrounds, may hold important insights for understanding the differences in the adaptive mechanisms between the two photoreceptor types.

Rods and cones differ considerably in the kinetics of their photoresponses [36, 37]. In general, the electrical responses from cones are three- to tenfold faster than the response from rods both in terms of time to peak and decay [38, 39]. However, differences also exist in the properties of responses among the various cone subtypes. In the salamander retina for instance, responses of violet cones exhibit more rod-like behavior, with slower kinetics and higher sensitivity than red cones [37, 40]. Similarly, green rods and blue cones in salamander share the same visual pigment and produce similar dark-adapted responses [41]. However, the properties of these two photoreceptors diverge in background light where they behave like typical rods and cones, respectively.

The two photoreceptors also differ in the way photoactivating, or bleaching, a fraction of the pigment affects their physiology. The decrease in the dark current after comparable bleaches is substantially smaller in cones [42] than in rods [43]. Recovery of responsiveness after exposure to bleaching light in the absence of 11-*cis*-retinal is faster in cones, where a steady state is reached in a few seconds, compared to rods, where over 30 min are required for a steady state. The recovery of sensitivity after treatment with 11-*cis*-retinal is also significantly faster in cones where dark adaptation occurs in the order of seconds [44]. In contrast, in rods, tens of minutes are required for complete dark adaptation [42, 45]. Light adaptation, on the other hand takes place in a matter of seconds in both rods and cones [42].

Consistent with these differences observed from isolated photoreceptors, rods and cones also differ in their respective kinetics of dark adaptation in vivo (Fig. 3.2) (reviewed in [46]). In fact, cone dark adaptation is so much faster that it was completely missed in an early study of dark adaptation and only the rod dark adaptation was observed [47]. Hecht and coworkers were the first to show that in humans, after exposure to very bright light, cones dark adapt several times faster than rods [29]. They observed that following the exposure to bright light, dark adaptation occurs in two parts. The first, which apparently begins at once, the authors attributed to cone function. The second part, which appeared later and was much slower, they attributed to rod function. In their experiments, cone dark adaptation was complete in 3–4 min, whereas rod dark adaptation took at least 30 min.

Recent biochemical studies indicate that one mechanism contributing to the faster dark adaptation of cones compared to rods is based on the supply of recycled

chromophore for pigment regeneration. The canonical visual cycle involves the pigment epithelium (Fig. 3.3), where all-*trans*-retinol is converted into 11-*cis*-retinal via a series of enzymatic reactions and then transported back to the photoreceptors for incorporation into opsin [48–51]. The rapid dark adaptation of cones and their ability to maintain adequate levels of pigment and remain light sensitive even in steady bright light require rapid pigment regeneration, whence rapid recycling of chromophore for cones. However, the slow rate of chromophore turnover in the pigment epithelium and the competition for recycled chromophore between cone opsin and overwhelming levels of rod opsin in most rod-dominant species indicate that the canonical retinal pigment epithelium (RPE) visual cycle might not be sufficient to meet the chromophore demand of cones. Indeed, recent biochemical studies from cone-dominant species have brought up the idea of a second, cone-specific pathway for recycling of chromophore located within the retina and possibly relying on the Müller cells (Fig. 3.3) [52–55]. The role of this novel cycle in mammalian rod-dominant species is still controversial. However, recent physiological experiments with amphibian [56], fish [57], and mammalian [58, 59] photoreceptors demonstrate the function of a cone-specific retina visual cycle under physiological conditions in a rod-dominant retina [15, 60]. Importantly, the combined action of the pigment epithelium and the retina visual cycles is required for the rapid and complete dark adaptation of cones.

Photochemical and Thermal Characteristics of Rod and Cone Pigments

Activation of the visual pigment by light is the first step in phototransduction and is subject to significant amplification. Thus, it is conceivable that differences in the properties of rod and cone visual pigments can impact substantially the functional properties of rods and cones. The quantum efficiency, defined as the ratio of the number of photoactivated visual pigment molecules to the number of such molecules that absorbed a photon, is the same in rods and cones [61] and equals to about 0.7 (see also [62]). Thus, in both photoreceptor types, roughly 70 % of the absorbed photons will trigger photoactivation of rhodopsin, with the remaining 30 % of the absorbed photons dissipated as heat.

The quantum efficiency for photoactivation can be expressed as the molar photosensitivity divided by the molar extinction coefficient. Thus, another, indirect way of comparing the quantum efficiency of rod and cone pigments is to compare their photosensitivities and their extinction coefficients. The extinction coefficients of three of the chicken pigments have been measured. Those include the red cone ($\varepsilon = 47{,}200$, [63]) and the green cone pigment ($\varepsilon = 40{,}800$, [64]), and the chicken rod pigment ($\varepsilon = 40{,}700$, [63]). Adding to the observation that these numbers are very similar, the fact that these pigments also have similar photosensitivities [64], one can once again conclude that rod and cone pigments possess comparable quantum efficiencies. Therefore, the differences in the sensitivities of rods and cones most

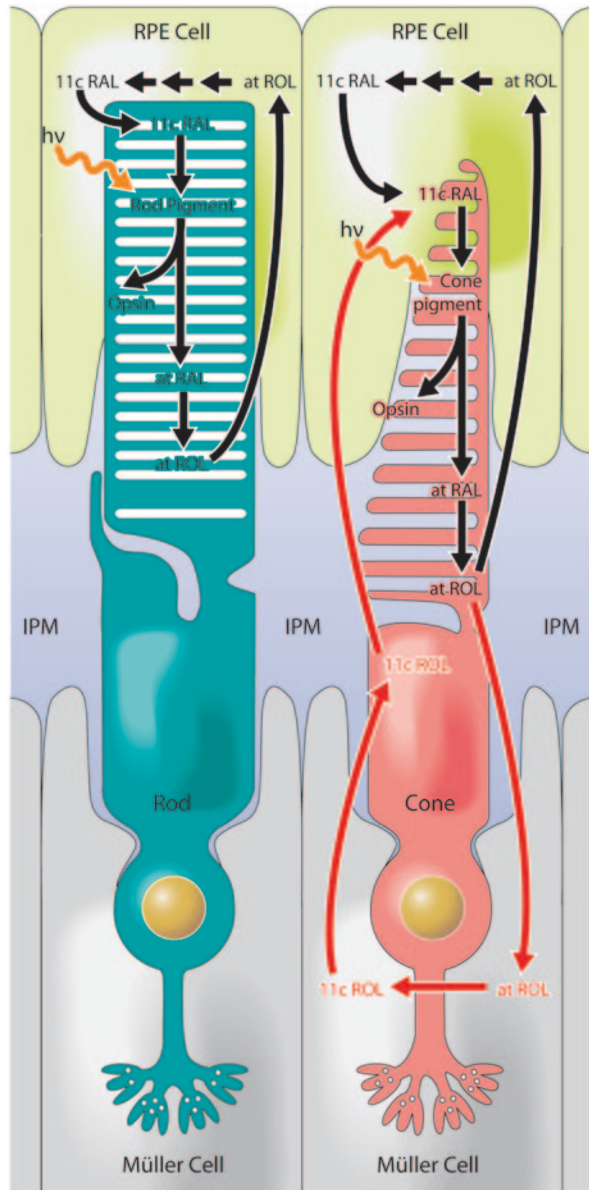

Fig. 3.3 The two visual cycles in the eye. The canonical retinal pigment epithelium (RPE) visual cycle (*black arrows*) recycles all-*trans*-retinol released from rods and cones following a bleach to 11-*cis*-retinal which can be used by both rods and cones for pigment regeneration. The retina visual cycle (*red arrows*) uses glial Müller cells to recycle all-*trans*-retinol to 11-*cis*-retinol, which only cones can oxidize to 11-*cis*-retinal for pigment regeneration. *RAL* retinal, *ROL* retinol, *11c* 11-*cis, at* all-*trans, IPM* interphotoreceptor matrix. (This figure was originally published in the Journal of Biological Chemistry [15] © the American Society for Biochemistry and Molecular Biology)

likely do not derive from the differences in the ability of their pigments to absorb photons and be activated.

Another long-standing hypothesis linking the visual pigment to the differences between rods and cones is that the thermal stability of rod pigments is higher than that of cone pigments [65, 66]. Indeed, studies with amphibian photoreceptors indicate that the different stability of rod and cone pigments modulate their respective

phototransduction cascades. First, analysis of transgenic *Xenopus* rods expressing red cone opsin has allowed the direct observation of physiological responses to the activation of a single cone pigment molecule [67]. This has made possible the determination of the rate of spontaneous thermal activation of red cone pigments, which produces a response identical to the activation by a photon [68]. The molecular rate of thermal activation measured in this way is ~10,000 times higher for red cone pigment than for rod pigment. As a result, amphibian red cones experience ~200 pigment activations per second in darkness. This level of dark activity is comparable to the total dark noise measured from salamander red cones [69] indicating that most of the noise in these cells originates from the thermal activation of the pigment. This spontaneous activity acts as background light to induce adaptation and, therefore, desensitization and acceleration of the flash response [70]. A second mechanism by which the stability of the visual pigment contributes to the differences between rods and cones is based on the covalent bond between opsin and retinal in their respective pigments. Both biochemical [71] and physiological [72] studies indicate that the formation of the covalent bond between opsin and chromophore is reversible in amphibian cones but not in rods. As a result, the visual pigment in cones, but not in rods, can spontaneously dissociate into free opsin and 11-*cis*-retinal. The very low level of free 11-*cis*-retinal in the outer segment (only ~0.1 % of the pigment content) shifts the equilibrium between free and chromophore-bound cone opsin so that even in dark-adapted cones there is ~10 % free opsin. At this high level, the total catalytic activity of free opsin, though weak per single molecule [73], is sufficient to induce adaptation and further reduce the sensitivity and accelerate the kinetics of the cone flash responses [72].

The effects of pigment properties on mammalian photoreceptor function have not been well characterized. Interestingly, studies from transgenic mouse rods expressing cone pigments indicate that, though still significantly higher than that of rod pigment, the rate of thermal activation of cone pigment is not high enough to affect significantly cone photosensitivity [74, 75]. A possible explanation for the relatively low thermal activity of cone pigments in mammalian species compared to amphibians might be that they use a slightly different chromophore (11-*cis*-retinal, or A1) than most amphibian photoreceptors (11-*cis*-3-dehydroretinal, or A2). The reversibility of cone pigment formation and its possible effect on cone function have not yet been examined in mammalian cones. Finally, differences in the properties of rod and cone visual pigments also contribute to the very different rates of dark adaptation in rods and cones discussed above.

Phototransduction in Rods and Cones

As already mentioned above, phototransduction in retinal rod and cone photoreceptor cells relies on the classic heterotrimeric G-protein-mediated pathway, which has served this important role based on more than three decades of intense research. Mechanistic details of coupling between corresponding visual pigments and the

intracellular enzyme of the photoreceptor second-messenger system, cGMP–PDE, are believed to be very similar and conserved among vertebrates [76]. Yet, rods and cones contain distinct sets of G proteins, which represent various isoforms of large and diverse mammalian protein families comprised of 16 Gα (including four subfamilies: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$), 5 Gβ and 12 Gγ subunits [77].

Both rods and cones rely on the inhibitory G_i class of G proteins. Rod transducin, the multisubunit enzyme of the phototransduction cascade (Fig. 3.1), is composed of the $G\alpha_{t1}$ subunit (39 kDa) and the obligate dimer, Gβγ subunit complex ($G\beta_1$, 36 kDa, and $G\gamma_1$, 8 kDa), which is dissociable only under denaturing conditions [78–80]. In contrast, cones contain $G\alpha_{t2}\beta_3\gamma_8$ transducin heterotrimers [81–84]. Rod and cone subunits of transducin share fairly high levels of amino acid identity: $G\alpha_{t1}$ is 78 % identical to $G\alpha_{t2}$, $G\beta_1$ is 80 % identical to $G\beta_3$, and $G\gamma_1$ is 64 % identical to $G\gamma_8$.

The subunits of transducin have distinct functional roles. The G protein α-subunits give the heterotrimeric G proteins its overall name as enzymes that possess GTP-binding and GTP-hydrolyzing properties. They are precise nucleotide-dependent switches that determine the amplification rate and the time course of the photoresponse. The type of the nucleotide bound to Gα controls the status of the switch, since it determines the overall conformation of Gα: the GDP-bound state is inactive and the GTP-bound form is the active state [85–88].

In rods, $G\alpha_{t1}$ is the exclusive signal transducer and amplifier. Deletion of the $G\alpha_{t1}$ gene (*Gnat1*) in mice completely eliminates scotopic vision [89]. Activation of the phototransduction cascade starts when the photoactivated conformation of rhodopsin, metarhodopsin II (Meta II, R*), characterized by the de-protonated Schiff-base linkage, catalyzes the exchange of the bound GDP with GTP on $G\alpha_t$ via a transient nucleotide-empty R*–G_t complex [90, 91]. The high efficiency of R*–G_t interaction is at the basis of the first step of signal amplification in the overall cascade of vision. During its lifetime, a single molecule of R* is thought to activate 20–100 molecules of G_t [92, 93], which ultimately results in the hydrolysis of 10^5 molecules of cGMP by PDE [94].

The lifetime of the active $G\alpha_t$–GTP is controlled by the GTPase activity of $G\alpha_t$. The basal GTPase activity is relatively slow but it is accelerated markedly when $G\alpha_t$ forms the G_t/PDE/RGS9 (Regulator of G Protein Signaling 9) complex. GTPase reaction is crucial for the overall timing of the photoresponse, as it has been shown to be rate limiting in controlling the overall rate of photoresponse recovery [95, 96]. The stereochemistry of nucleotide binding and hydrolysis of the monomeric and heterotrimeric G proteins as well as Gα conformations have been reviewed [97–100].

While the physiological functions of $G\alpha_t$ are now fairly well understood, the molecular and physiological roles of transducin Gβγ continue to draw significant interest. It is the involvement of Gβγ subunit complex that makes heterotrimeric G proteins distinct from the small monomeric G proteins. It was originally purified as part of G_t from bovine rods by an ingenious and now widely used procedure, which utilizes highly enriched rod outer segment (ROS) membranes [101], and high-affinity binding of G_t to R* with subsequent release of G_t in low ionic strength buffer containing GTP [85, 102, 103].

All major steps of the Gα_t activation cycle require the Gβγ complex [104]. The cycle starts with the inactive G_t–GDP. Two posttranslational lipid modifications, at the N-terminus of Gα_t by the heterogeneous mixture of fatty acids (C<14) [105], and at the C-terminus of Gγ by the isoprenoid farnesyl (C=15) [106], contribute to the lipid–lipid and lipid–protein interactions [107]. Overall, G_t heterotrimer is the least hydrophobic among heterotrimeric G proteins, which makes it easily soluble in biological buffers containing no detergents, and determines its unique membrane/cytosol partitioning properties inside the photoreceptor cells [107]. The weaker lipid membrane interactions of the farnesylated Gβγ, compared to the geranylgeranylated Gβγ subtypes in other cells, appear to be crucial for proper translocation of Gβγ within the rod photoreceptor cell and the mechanism of light adaptation [108].

The hydrophobic properties of Gβγ regulate the membrane attachment of the heterotrimer [109], which is essential for the high rate of G_t activation by R* [92]. One of the first identified roles for Gβγ was in fact to facilitate G_t targeting to the lipid membranes, binding to the photoactivated rhodopsin [103, 110], and facilitation of R*–G_t complex formation via enhanced collision coupling [90], which results in higher rates of rhodopsin-catalyzed nucleotide exchange. For many years this conclusion relied solely on biochemical data, until Gβγ was eliminated from mouse rods recently by the genetic deletion of Gγ_1 gene (*Gngt1*). One of the major difficulties of calculating signal amplification in photoreceptors, which is proportional to the rate of Gα_t activation by R*, after complete or partial removal of Gβγ in native rods is that it can be obscured by the concomitant reduction of Gα_t concentration. Thus, careful analysis of the membrane-bound fraction of Gα_t in ROS is necessary. Physiological and biochemical analysis of the Gγ_1-deficient mice confirmed that rods require Gβγ for efficient amplification of the visual signal. The reduction of signal amplification at the R*–G_t interaction step was a significant contributing factor to the overall 90-fold reduction of scotopic light sensitivity in the absence of Gγ_t ([111], see also [112]). Interestingly, desensitization of mouse cones after the targeted deletion of Gβ_3 was concluded to be proportional to the reduction of Gα_{t2} [113] indicating either potential lack of modulation of signal amplification by the cone G$\beta_3\gamma_8$ complex or possible compensatory effects from other Gβγ complexes. Whether this represents a meaningful difference between rod G$\beta_1\gamma_1$ and cone G$\beta_3\gamma_8$ transducin complexes or signaling via other redundant G proteins in cones remains to be elucidated.

The answer to the mechanistic puzzle of how R* catalyzes the nucleotide exchange on Gα_t undoubtedly centers on the elusive R*–G_t nucleotide empty complex. While transient in rods under physiological conditions because of the high cytoplasmic GTP concentration, the complex is stable when formed in vitro under conditions of no nucleotide present [91]. Significant research effort is underway to determine the high-resolution structure of the complex and the conformational changes that accompany signal transfer from R* to G_t, reviewed in [100, 114, 115]. Currently, the crystal structure of the β_2-adrenergic receptor monomer/G_s complex [116], which is another prototypical GPCR system that is thought to share the mechanisms of activation with the visual system, is the best approximation of the R*–G_t interactions. The visual system, however, continues to provide crucial insights on

the mechanism of activation by broadening the scope of the GPCR/G protein model to include rhodopsin oligomers [117].

One of the fundamental features of R* is its ability to act catalytically on many G_t–GDP molecules. Upon activation, $G\alpha_t$–GTP and Gβγ dissociate from each other and from R*. The dissociation of Gβγ is essential for production of free $G\alpha_t$–GTP, which is capable of interacting with and relieving the action of the inhibitory PDEγ subunit, because the sites of interactions of $G\alpha_t$ with Gβγ and PDEγ have significant steric overlap [118]. Whether Gβγ truly dissociates from R* during multiple activation cycles in vivo, or remains bound to Meta II and regulates its photochemical properties or lifetime was examined recently using a mouse model lacking rod $G\gamma_1$. Consistent with the earlier biochemical and physiological data, complex formation between photoactivated rhodopsin and transducin is severely compromised in the absence of Gβγ. The photoactivation and thermal photoproduct decay, however, remained unchanged, which supports the model of Gβγ dissociation from R* [119].

Finally, after GTP hydrolysis, the last step of the G protein cycle is marked by the reassociation of $G\alpha_t$–GDP with Gβγ to form the inactive form of transducin heterotrimer, ready for the next activation cycle. At this step, Gβγ acts as a typical guanine nucleotide dissociation inhibitor (GDI) [120]. Binding of Gβγ also occludes the effector interacting sites on $G\alpha_t$–GDP, which further stabilizes the inactive conformation of the heterotrimer and prevents accidental activation of PDE.

Contribution of Transducin to the Functional Differences Between Rods and Cones

As discussed above, mammalian rod photoreceptors are 100–1000-fold more sensitive to light compared to cones. It is likely that multiple mechanisms contribute to this important functional difference. A number of previous studies, however, suggest that one of the major mechanisms could be the reduced signal amplification rate in cones [40, 121, 122]. Considering the key role of G_t in controlling signal amplification, one of the main factors to explain the lower amplification and photosensitivity of cones could be the unique functional properties of cone-specific isoforms of G_t. Not surprisingly, the extent of contribution of the distinct isoforms of transducin subunits to rod/cone physiological differences has been a matter of intensive research efforts.

Rod and cone G_t heterotrimers are considered unique and the sole signal transducers in rods and cones respectively, compared to other cell types that contain multiple G protein isoforms. Thus, replacing individual subunits in retinal photoreceptors is a powerful approach to address their functional differences. Each of the three subunits of transducin: $G\alpha_{t1}$ versus $G\alpha_{t2}$, $G\beta_1$ *vs.* $G\beta_3$, and $G\gamma_1$ *vs.* $G\gamma_8$ can potentially contribute to the observed lower rate of G_t activation in cones.

Analysis of recent reports that investigated the functional interchangeability between the rod and cone $G\alpha_t$ subunits reveal somewhat conflicting results with regard to the physiological consequences of substituting cone $G\alpha_{t2}$ for rod $G\alpha_{t1}$ in

mouse rods. The first evidence came from the transient expression of cone $G\alpha_{t2}$ in mouse rods and the reciprocal expression of rod $G\alpha_{t1}$ in cones, using the adeno-associated virus (AAV) technology. The results obtained from transretinal ERGs and single cell recordings showed that although protein sequences of rod and cone $G\alpha_t$ are only 78% identical, the proteins are functionally interchangeable [123]. This work was followed by the stable transgenic expression of cone $G\alpha_{t2}$ in mouse rods lacking the endogenous rod $G\alpha_{t1}$. Physiological analysis of this model showed that cone $G\alpha_{t2}$ expressed in rods resulted in significantly decreased photosensitivity, reduced rate of activation, and accelerated recovery that are typical of cone photoreceptors [124]. Finally, a recent study reported transgenic expression of cone $G\alpha_{t2}$ in mouse rods on the rod $G\alpha_{t1}$-deficient background and also expressing S-opsin, in order to compare the amplification rates from different GPCRs in the same cell. Single cell recordings revealed that rod and cone $G\alpha_t$ couple to rhodopsin and cone S-opsin with similar efficiency [125]. Interestingly, biochemical analysis of purified proteins aimed at addressing $G\alpha_{t1}/G\alpha_{t2}$ differences demonstrated that, while spontaneous nucleotide exchange on $G\alpha_{t2}$ was about tenfold higher, the rate of R*-catalyzed activation as well as the rate of inactivation for the rod and cone isoforms of $G\alpha_t$ were identical [126].

Overall, the majority of the data point to close functional similarity and good interchangeability between $G\alpha_{t1}$ and $G\alpha_{t2}$. Thus, the lower visual sensitivity of cones compared to rods and reduced rate of signal transduction between the cone visual pigment and PDE cannot be explained by the differences in $G\alpha_t$ subunits. Similar studies are underway to address the possible role of the rod and cone transducin Gβγ complexes in this regard [127].

Assembly of Heterotrimeric Transducin in Rod Cells

To carry out its signaling role in phototransduction, the functional transducin heterotrimer should first be formed from its individual subunits. As mentioned above, the rod and cone photoreceptor transducins have different subunit compositions [84], which is $G\alpha_{t1}\beta_1\gamma_1$ in rods and $Gt\alpha_2\beta_3\gamma_8$ in cones. The machinery for synthesis and assembly of both $G\alpha_{t1}$ and $G\beta_1\gamma_1$ subunits is located in the photoreceptor inner segment and involves multiple auxiliary proteins and organelles. While the mechanisms of transducin assembly have become increasingly appreciated, until recently most of our knowledge of this process was derived from studies of rod $G\beta_1\gamma_1$ complex formation either *in vitro* or in transfected cells expressing recombinant G_t subunits [128]. In addition, conclusions about photoreceptor transducin were often extrapolated from results obtained with different Gβγ compositions, such as the most common $G\beta_1\gamma_2$ dimer [129–132]. Like all other known cellular Gβγ complexes that can be formed in principle, mature $G\beta_1\gamma_1$ is an obligate heterodimer in which both $G\beta_1$ and $G\gamma_1$ constituents are unstable on their own [133–135] and must be associated together to avoid proteolytic degradation.

During the last decade, several accessory proteins for G_t folding have been identified. It has been established that, among other Gβγ dimer combinations, the assembly of the rod $G\beta_1\gamma_1$ complex critically depends on the molecular chaperone CCT (chaperonin-containing tailless polypeptide 1, also known as TRiC [136]). CCT is a large cytosolic chaperonine protein (~ 1 MDa) of cylindrical shape formed by a pair of precisely stacked rings. Each ring consists of eight different 60 kDa subunits, denoted as CCT_α–CCT_θ in mammals that enclose a central CCT cavity [137–140]. Each CCT subunit has adenosine triphosphate (ATP)-binding site and possesses ATPase activity required for transition of CCT to its closed conformation in which nascent polypeptide chain of CCT substrate is folded [137, 141, 142]. The CCT complex is present in rod photoreceptors [143–145] where its content reaches ~ 65 % of total CCT in the retina [146]. A recent study of transgenic disruption of CCT function in mouse rods provided the first demonstration of its significance in mammalian photoreceptors in vivo. This manipulation affected protein networks that are essential for rod outer segment morphogenesis, viability, and Gt signaling, and has been shown to lead to rapid death of rods and cause progressive retinal degeneration [147].

The proposed mechanism of $G\beta_1$ folding on CCT chaperonine is schematically shown in Fig. 3.4, along with the entire sequence of G_t heterotrimer formation. Newly ribosome-translated nascent $G\beta_1$ is delivered to CCT by yet uncharacterized mechanism, enters CCT internal cavity, and is folded there in an ATP-dependent manner adopting its stable seven-bladed β-propeller conformation. Unfolded $G\beta_1$ becomes a subject for intracellular aggregation and degradation [148, 149]. However, CCT alone is insufficient to assemble $G\beta_1\gamma_1$ dimer in vivo; this process also requires the co-chaperone called phosducin-like protein 1 (PhLP1) [150], a member of the phosducin family of proteins having ~65 % homology to phosducin [151]. PhLP1 is highly expressed in rod photoreceptors and other retinal neurons [144, 150, 152]. It binds CCT [129, 130, 153], where it occupies the apical part of one of the two CCT rings and thus blocks the access for other CCT substrate molecules to its central chamber [154] while further assisting $G\beta_1$ folding. The stability of the resulting PhLP1/CCT/$G\beta_1$ ternary complex depends on phosphorylation status of PhLP1 which is maintained by caseine kinase 2 (CK2). The N-terminal phosphorylation of PhLP1 (at serine 18–20 cluster) causes the release of PhLP1/$G\beta_1$ complex from CCT [131]. However, it is currently unclear when phosphorylation takes place and whether dephosphorylation events occur, since this type of posttranslational modification may be constitutive in many tissues in vivo [155, 156].

Once released from CCT, PhLP1/$G\beta_1$ rapidly interacts with a prefolded $G\gamma_1$ subunit to complete the assembly of $G\beta_1\gamma_1$ dimer and disengage PhLP1 which becomes ready to assist a new round of CCT-assisted $G\beta_1$ folding [136, 157]. An important insight into the process of PhLP1-dependent assembly of $G\beta_1\gamma_1$ in rod photoreceptors and its impact on phototransduction has been recently obtained from a mouse model with conditionally ablated PhLP1 [150]. The lack of PhLP1 had profound effects on G_t heterotrimer formation in mouse rods, resulting in accumulation of unassembled $G\beta_1$ on CCT, the deficiency in biogenesis of both $G\alpha_{t1}$ and $G\gamma_1$ subunits, substantial desensitization of photoreceptors, and severely compromised scotopic

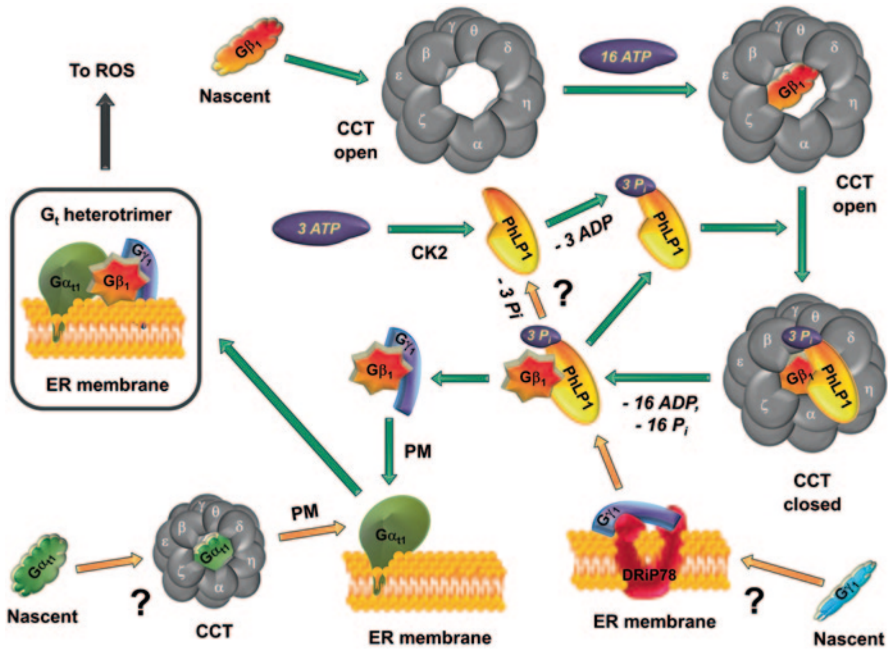

Fig. 3.4 The formation of $G\alpha_{t1}\beta_1\gamma_1$ transducin heterotrimer in the inner segment of rod photoreceptors is assisted by molecular chaperones chaperonin-containing tailless polypeptide 1 (*CCT*), phosducin-like protein 1 (*PhLP1*), and (presumably) dopamine receptor-interacting protein 78 (*DRiP78*)-like protein. See the text for specific details. Other designations in the scheme: *ROS* rod outer segment, *CK2* caseine kinase 2, *ER* endoplasmic reticulum, *PM* posttranslational lipid modifications of $G\alpha_{t1}$ and $G\gamma_1$, *ATP* adenosine triphosphate, *ADP* adenosine diphosphate, *Pi* inorganic phosphate

vision [150]. The same study also demonstrated the involvement of PhLP1 in the assembly of another rod outer segment-signaling complex, RGS9/Gβ_5, which is required for timely inactivation of $G\alpha_{t1}$–GTP/PDE and termination of the photoresponse [150].

Even a more severe degeneration of rods has been documented in transgenic mice expressing the dominant-negative $^{\Delta 1-83}$PhLP1 mutant lacking its N-terminus including S18–20 phosphorylation site, with identical underlying mechanism of retention of unfolded Gβ_1 on CCT [147] and thus preventing the processing of multiple cytosolic protein substrates of CCT [158, 159]. In addition, the overexpression of this mutant, also known as PhLPs, which is a product of alternative splicing of PhLP1 gene in human retina [160] but is normally absent in mice, caused strong suppression of the synthesis of $G\alpha_{t1}$ subunit at transcriptional level suggesting that it may be the first identified negative regulator of assembly of heterotrimeric G_t in vivo [157]. However, considering the lower physiological level of its expression than that of full-length PhLP1 [160], its potential CCT modulatory function in human rods remains to be elucidated.

The expression levels of $G\alpha_{t1}$ and $G\beta_1$ critically depend also on $G\gamma_1$ subunit whose expression is required for maintaining their proteolytic stability inside the cell. This conclusion stems from the fact that in two separate $G\gamma_1$-deficient mouse lines both $G\alpha_{t1}$ and $G\beta_1$ were markedly reduced in ROS (although to a different degree), even before the onset of progressive retinal degeneration observed in these mice [111, 112], which likely resulted from gross proteosomal overload with unfolded $G\beta_1$ jammed on CCT [146]. Despite these in vivo observations, still very little is known about the processing of $G\gamma_1$ subunit after the exit of its nascent polypeptide from the ribosome (Fig. 3.4). $G\gamma_1$ is not one of CCT folding substrates since it does not associate with CCT in cell-free translation systems [136]. It has been suggested that endoplasmic reticulum (ER) resident dopamine receptor-interacting protein 78 (DRiP78, or DnaJ homolog subfamily C member 14) serves as molecular chaperone for $G\gamma_2$ before its association with $G\beta_1$ [161], presumably operating as C-terminally linked dimer [162, 163]. DRiP78 is a two-transmembrane domain HSP40-like protein containing J-domain that is required to enhance the intrinsic ATPase activity of chaperone HSP70 whose isoforms are also present in photoreceptors [143, 145]. It can directly interact with PhLP1/$G\beta_1$ in transfected cells and facilitate the binding of newly synthesized Gγ to this complex thus preventing the subunit from degradation [161]. For $G\gamma_1$, the degradation process is believed to involve the 26S proteasome following ubiquitylation [148]. The association of $G\gamma_1$ with PhLP1/$G\beta_1$ occurs on the opposite side of $G\beta_1$, not occupied by PhLP1, as follows from the resolved phosducin–$G\beta_1\gamma_1$ structure [164].

However, despite documented localization of dopamine D2 and D4 receptors in photoreceptor inner segments [165–168], the expression of the dopamine receptor-interacting protein DRiP78 in photoreceptors has not been reported. Furthermore, DRiP78 has the lowest capacity to interact with $G\gamma_1$ among other Gγ subunits tested in cell culture experiments [161]. Interestingly, proteomic and immunohistochemical (IHC) data show the presence of cysteine string protein α (CSPα) in bovine [143] and mouse [169] rod outer segments, in addition to its abundance in synaptic terminals. This protein, also known as DnaJ homolog C5 in humans, contains DnaJ-domain, which is a characteristic of Hsp40 type co-chaperones, and can bind G protein α-subunits [170]. CSPα deficiency causes massive and rapid photoreceptor degeneration of mostly synaptic origin [169], but whether CSPα or other photoreceptor chaperones detected in proteomic analyses [143, 145] are also involved in G_t assembly is currently unclear.

The C-terminal "CAAX" motif of $G\gamma_1$ subunit in complex with $G\beta_1$ is further posttranslationally prenylated with the isoprenoid farnesyl, by the enzyme farnesyltransferase (FTase) [106, 171] that presumably occurs near the ER membrane. Subsequent removal of the AAX sequence by the Ras-converting enzyme 1 (Rce1)-mediated proteolysis [172, 173] and the final carboxyl methylation of the C-terminal cysteine of $G\gamma_1$ by isoprenylcysteine carboxyl methyltransferase (ICMT) [174–176] complete the posttranslational modifications of $G\beta_1\gamma_1$ complex, presumably at the cytoplasmic surface of the ER [177]. These modifications greatly enhance the affinity of $G\beta_1\gamma_1$ to membrane lipids and also facilitate its interaction with $G\alpha_1$ subunit [176, 178, 179].

The association of $G\beta_1\gamma_1$ with $G\alpha_{t1}$ is the final step of G_t assembly (Fig. 3.4). However, the mechanism of $G\alpha_{t1}$ processing is sparsely explored. Comparison of crystal structures of phosducin/$G\beta_1\gamma_1$ [164, 180] and heterotrimeric G_t [86] shows that phosducin N-terminal domain and $G\alpha_{t1}$ occupy the same surface on $G\beta_1\gamma_1$, therefore PhLP1 should be displaced from $G\beta_1\gamma_1$, perhaps by $G\alpha_{t1}$, which also has an increased affinity to lipid membranes. The affinity is provided by heterogeneous N-acylation of $G\alpha_{t1}$ with C12:0, C14:0, C14:1, and C14:2 fatty acid moieties [105, 181] by still to be determined acyltransferase. The absence of the myristoyl group on $G\alpha_{t1}$ in $G\alpha_{t1}$-G2A mutant results in its permanent mislocalization to the inner segments of rod photoreceptors and leads to markedly diminished phototransduction [182].

It has been suggested that CCT chaperonin is also involved in the folding of $G\alpha_{t1}$ [183]. In that study, CCT was co-immunoprecipitated with not-yet-native $G\alpha_{t1}$ subunit in either cell-free translation system or rat rod photoreceptor culture and, like in the case of other CCT substrates, the release of folded functional $G\alpha_{t1}$ from CCT was stimulated by ATP. Recent in vivo studies clearly demonstrated the remarkable downregulation of $G\alpha_{t1}$ level in rods with compromised CCT function caused by latching of unfolded $G\beta_1$ subunit in CCT binding pocket, even in predegenerated cells [111, 112, 150]. Yet, the direct physiological evidence of CCT-mediated $G\alpha_{t1}$ folding is still missing, since the possibility of secondary loss of the bulk of $G\alpha_{t1}$ that could be initially processed by other cellular chaperones in the absence of $G\beta_1\gamma_1$ in those mouse models cannot be ruled out. Toward this end, a number of other potential chaperones including Ric-8 [184–187] and HSP70/90 [188, 189] have been suggested to assist the folding of nontransducin Gα subunits in various model systems. Modern proteomic approaches [143, 145] and IHC techniques [144, 190, 191] reveal the presence of HSP70/90 chaperones in photoreceptors, and their potential role in $G\alpha_{t1}$ folding and $G\alpha_{t1}\beta_1\gamma_1$ assembly is an exciting area of future investigation. Finally, using genetic manipulations and new animal models will help to unravel the mechanisms of chaperone-assisted assembly and formation of $G\alpha_{t2}\beta_3\gamma_8$ heterotrimer in cone photoreceptor cells.

Eventually, G_t is trafficked from the ER membrane [177] to its final destination, the photoreceptor outer segment. There is substantial evidence supporting the idea that the bulk of G_t is transported in the form of fully assembled heterotrimer, since in dark-adapted rods of $G\gamma_1$ knock-out mice $G\alpha_{t1}$ is evenly distributed through all photoreceptor compartments, and the same mislocalization is observed for $G\beta_1\gamma_1$ in dark-adapted $G\alpha_{t1}$ knock-out mice [112, 192]. However, more recent findings have challenged this idea and suggested that significant fraction of heterotrimeric G_t on rod inner segment (RIS) ER membrane can be dissociated to its constituents by uncoordinated 119 (UNC119) and prenyl binding (PrBP/δ) proteins that are then involved in presumably independent transport to the outer segment of $G\alpha_{t1}$ and $G\beta_1\gamma_1$ subunits, respectively [193–198]. In addition, the delivery of $G\beta_1\gamma_1$ to ROS is facilitated by phosducin [199]. In the outer segment, G_t heterotrimer is reassembled, anchored on the cytosolic side of disc membranes by its lipid moieties, and becomes ready to perform its major function of signaling in the phototransduction cascade.

Summary

Biochemical and physiological studies of retinal rod and cone photoreceptors have been leading the way in understanding the quantitative details of phototransduction and G protein signaling in general. With the advancement of new genetic and molecular tools, the road ahead looks exciting and should produce further insight of the G protein signaling and its role in all aspects of human biology.

References

1. Arshavsky VY, Lamb TD, Pugh EN Jr (2002) G proteins and phototransduction. Annu Rev Physiol 64:153–187
2. Burns ME, Baylor DA (2001) Activation, deactivation, and adaptation in vertebrate photoreceptor cells. Annu Rev Neurosci 24:779–805
3. Ebrey T, Koutalos Y (2001) Vertebrate photoreceptors. Prog Retin Eye Res 20:49–94
4. Fu Y, Yau K-W (2007) Phototransduction in mouse rods and cones. Pflugers Arch 454:805–819
5. Nathans J (1987) Molecular biology of visual pigments. Annu Rev Neurosci 10:163–194
6. Hargrave PA (2001) Rhodopsin structure, function, and topography the Friedenwald lecture. Invest Ophthalmol Vis Sci 42:3–9
7. Wald G (1949) The photochemistry of vision. Documenta Ophthalmol 3:94–137
8. Matsumoto H, Yoshizawa T (1975) Existence of a beta-ionone ring-binding site in the rhodopsin molecule. Nature 258:523–526
9. Wald G (1935) Carotenoids and the visual cycle. J Gen Physiol 19:351–371
10. Wald G (1935) Vitamin a in eye tissues. J Gen Physiol 18:905–915
11. Bownds D (1967) Site of attachment of retinal in rhodopsin. Nature 216:1178–1181
12. Palczewski K (2006) G protein-coupled receptor rhodopsin. Annu Rev Biochem 75:743–767
13. Cornwall MC, Jones GJ, Kefalov VJ, Fain GL, Matthews HR (2000) Electrophysiological methods for measurement of activation of phototransduction by bleached visual pigment in salamander photoreceptors. Methods Enzymol 316:224–252
14. Crouch RK, Kefalov V, Gartner W, Cornwall MC (2002) Use of retinal analogues for the study of visual pigment function. Methods Enzymol 343:29–48
15. Kefalov VJ (2012) Rod and cone visual pigments and phototransduction through pharmacological, genetic, and physiological approaches. J Biol Chem 287:1635–1641
16. Kefalov VJ, Cornwall MC, Fain GL (2010) Physiological studies of the interaction between opsin and chromophore in rod and cone visual pigments. Methods Mol Biol 652:95–114
17. Palczewski K (2010) Retinoids for treatment of retinal diseases. Trends Pharmacol Sci 31:284–295
18. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM et al (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 358:2240–2248
19. Insinna C, Daniele LL, Davis JA, Larsen DD, Kuemmel C, Wang J, Nikonov SS, Knox BE, Pugh EN Jr (2012) An S-opsin knock-in mouse (F81Y) reveals a role for the native ligand 11-cis-retinal in cone opsin biosynthesis. J Neurosci 32:8094–8104
20. Kono M, Crouch RK (2011) Probing human red cone opsin activity with retinal analogues. J Nat Prod 74:391–394
21. Baylor DA, Lamb TD, Yau KW (1979) Responses of retinal rods to single photons. J Physiol 288:613–634
22. Hecht S, Shlaer S, Pirenne MH (1942) Energy, quanta, and vision. J Gen Physiol 25:819–840

23. Rushton WA (1965) The sensitivity of rods under illumination. J Physiol 178:141–160
24. Rushton WAH (1961) Rhodopsin measurement and dark-adaptation in a subject defficient in cone vision. J Physiol 156:193–205
25. Weale RA (1961) Limits of human vision. Nature 191:471–473
26. Fein A, Szuts E (1982) Photoreceptors: their role in vision, 1st edn. Cambridge University Press, Cambridge
27. Kleinschmidt J, Dowling JE (1975) Intracellular recordings from gecko photoreceptors during light and dark adaptation. J Gen Physiol 66:617–648
28. Williams TP, Webbers JP, Giordano L, Henderson RP (1998) Distribution of photon absorption rates across the rat retina. J Physiol (Lond) 508:515–522
29. Hecht S, Haig C, Chase AM (1937) Rod and cone adaptation. J Gen Physiol 20:831–850
30. Rushton WAH (1965) A foveal pigment in the deuteranope. J Physiol 176:24–37
31. Wald G, Brown PK, Smith PH (1955) Iodopsin. J Gen Physiol 38:623–681
32. Baylor DA, Hodgkin AL (1974) Changes in time scale and sensitivity in turtle photoreceptors. J Physiol (Lond) 242:729–758
33. Boynton RM, Whitten DN (1970) Visual adaptation in monkey cones: recordings of late receptor potentials. Science 170:1423–1426
34. Dowling JE, Ripps H (1972) Adaptation in skate photoreceptors. J Gen Physiol 60:698–719
35. Cornwall MC, Ripps H, Chappell RL, Jones GJ (1989) Membrane current responses of skate photoreceptors. J Gen Physiol 94:633–647
36. Miller JL, Picones A, Korenbrot JI (1994) Differences in transduction between rod and cone photoreceptors: an exploration of the role of calcium homeostasis. Curr Opin Neurobiol 4:488–495
37. Perry RJ, McNaughton PA (1991) Response properties of cones from the retina of the tiger salamander. (Published erratum appears in J Physiol (Lond) 1991 May;436:771). J Physiol (Lond) 433:561–587
38. Baylor D (1992) Transduction in retinal photoreceptor cells. Soc Gen Physiol Ser 47:151–174
39. Nakatani K, Yau KW (1988) Calcium and light adaptation in retinal rods and cones. Nature 334, 69–71
40. Nikonov SS, Kholodenko R, Lem J, Pugh EN Jr (2006) Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. J Gen Physiol 127:359–374
41. Ma J, Znoiko S, Othersen KL, Ryan JC, Das J, Isayama T, Kono M, Oprian DD, Corson DW, Cornwall MC et al (2001) A visual pigment expressed in both rod and cone photoreceptors. Neuron 32:451–461
42. Jones GJ, Crouch RK, Wiggert B, Cornwall MC, Chader GJ (1989) Retinoid requirements for recovery of sensitivity after visual-pigment bleaching in isolated photoreceptors. Proc Natl Acad Sci U S A 86:9606–9610
43. Jin J, Crouch RK, Corson DW, Katz BM, MacNichol EF, Cornwall MC (1993) Noncovalent occupancy of the retinal-binding pocket of opsin diminishes bleaching adaptation of retinal cones. Neuron 11:513–522
44. Kefalov VJ, Crouch RK, Cornwall MC (2001) Role of noncovalent binding of 11-cis-retinal to opsin in dark adaptation of rod and cone photoreceptors. Neuron 29:749–755
45. Pepperberg DR, Brown PK, Lurie M, Dowling JE (1978) Visual pigment and photoreceptor sensitivity in the isolated skate retina. J Gen Physiol 71:369–396
46. Lamb TD, Pugh EN Jr (2004) Dark adaptation and the retinoid cycle of vision. Prog Retin Eye Res 23:307–380
47. Piper H (1903) Uber Dunkeladaptation. Z Psychol Physiol Sinnesorg 31:161
48. Saari JC (2012) Vitamin A metabolism in rod and cone visual cycles. Ann Rev Nutr 32:125–145
49. Fain GL, Matthews HR, Cornwall MC, Koutalos Y (2001) Adaptation in vertebrate photoreceptors. Physiol Rev 81:117–151

50. Fain GL, Matthews HR, Cornwall MC (1996) Dark adaptation in vertebrate photoreceptors. Trends Neurosci 19:502–507
51. Kiser PD, Golczak M, Palczewski K (2013) Chemistry of the retinoid (visual) cycle. Chem Rev 114:194–232
52. Mata NL, Radu RA, Clemmons RC, Travis GH (2002) Isomerization and oxidation of vitamin A in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. Neuron 36:69–80
53. Bustamante JJ, Ziari S, Ramirez RD, Tsin AT (1995) Retinyl ester hydrolase and the visual cycle in the chicken eye. Am J Physiol 269:R1346–R1350
54. Das SR, Bhardwaj N, Kjeldbye H, Gouras P (1992) Muller cells of chicken retina synthesize 11-cis-retinol. Biochem J 285(Pt 3):907–913
55. Kaylor JJ, Yuan Q, Cook J, Sarfare S, Makshanoff J, Miu A, Kim A, Kim P, Habib S, Roybal CN et al (2013) Identification of DES1 as a vitamin A isomerase in Muller glial cells of the retina. Nat Chem Biol 9:30–36
56. Wang JS, Estevez ME, Cornwall MC, Kefalov VJ (2009) Intra-retinal visual cycle required for rapid and complete cone dark adaptation. Nat Neurosci 12:295–302
57. Fleisch VC, Neuhauss SC (2010) Parallel visual cycles in the zebrafish retina. Prog Retin Eye Res 29:476–486
58. Wang JS, Kefalov VJ (2009) An alternative pathway mediates the mouse and human cone visual cycle. Curr Biol 19:1665–1669
59. Kolesnikov AV, Tang PH, Parker RO, Crouch RK, Kefalov VJ (2011) The mammalian cone visual cycle promotes rapid M/L-cone pigment regeneration independently of the interphotoreceptor retinoid-binding protein. J Neurosci 31:7900–7909
60. Wang JS, Kefalov VJ (2011) The cone-specific visual cycle. Prog Retin Eye Res 30:115–128
61. Dartnall HJA (1972) Photosensitivity. In: Dartnall HJA (ed) Handbook of sensory physiology, vol VII/I. Springer, Berlin, pp. 122–145
62. Bowmaker JK, Dartnall HJ (1980) Visual pigments of rods and cones in a human retina. J Physiol (Lond) 298:501–511
63. Okano T, Fukada Y, Shichida Y, Yoshizawa T (1992) Photosensitivities of iodopsin and rhodopsins. Photochem Photobiol 56:995–1001
64. Shichida Y, Imai H, Imamoto Y, Fukada Y, Yoshizawa T (1994) Is chicken green-sensitive cone visual pigment a rhodopsin-like pigment? A comparative study of the molecular properties between chicken green and rhodopsin. Biochemistry 33:9040–9044
65. Barlow H (1956) Retinal noise and absolute threshold. J Opt Soc Am 46:634–639
66. Donner K (1992) Noise and the absolute thresholds of cone and rod vision. Vision Res 32:853–866
67. Kefalov V, Fu Y, Marsh-Armstrong N, Yau KW (2003) Role of visual pigment properties in rod and cone phototransduction. Nature 425:526–531
68. Sampath AP, Baylor DA (2002) Molecular mechanism of spontaneous pigment activation in retinal cones. Biophys J 83:184–193
69. Rieke F, Baylor DA (2000) Origin and functional impact of dark noise in retinal cones. Neuron 26:181–186
70. Luo D-G, Yue WWS, Ala-Laurila P, Yau K-W (2011) Activation of visual pigments by light and heat. Science 332:1307–1312
71. Matsumoto H, Tokunaga F, Yoshizawa T (1975) Accessibility of the iodopsin chromophore. Biochim Biophys Acta 404:300–308
72. Kefalov VJ, Estevez ME, Kono M, Goletz PW, Crouch RK, Cornwall MC, Yau KW (2005) Breaking the covalent bond-a pigment property that contributes to desensitization in cones. Neuron 46:879–890
73. Cornwall MC, Fain GL (1994) Bleached pigment activates transduction in isolated rods of the salamander retina. J Physiol 480(Pt 2):261–279
74. Fu Y, Kefalov V, Luo DG, Xue T, Yau KW (2008) Quantal noise from human red cone pigment. Nat Neurosci 11:565–571

75. Shi G, Yau KW, Chen J, Kefalov VJ (2007) Signaling properties of a short-wave cone visual pigment and its role in phototransduction. J Neurosci 27:10084–10093
76. Stryer L (1986) Cyclic GMP cascade of vision. Ann Rev Neurosci 9:87–119
77. Downes GB, Gautam N (1999) The G protein subunit gene families. Genomics 62:544–552
78. Gautam N, Downes GB, Yan K, Kisselev O (1998) The G-protein betagamma complex. Cell Signal 10:447–455
79. McIntire WE (2009) Structural determinants involved in the formation and activation of G protein betagamma dimers. Neurosignals 17:82–99
80. Burns ME, Arshavsky VY (2005) Beyond counting photons: trials and trends in vertebrate visual transduction. Neuron 48:387–401
81. Lee RH, Lieberman BS, Yamane HK, Bok D, Fung BK (1992) A third form of the G protein beta subunit. 1. Immunochemical identification and localization to cone photoreceptors. J Biol Chem 267:24776–24781
82. Lerea CL, Somers DE, Hurley JB, Klock IB, Bunt-Milam AH (1986) Identification of specific transducin alpha subunits in retinal rod and cone photoreceptors. Science 234:77–80
83. Ong OC, Yamane HK, Phan KB, Fong HK, Bok D, Lee RH, Fung BK (1995) Molecular cloning and characterization of the G protein gamma subunit of cone photoreceptors. J Biol Chem 270:8495–8500
84. Peng YW, Robishaw JD, Levine MA, Yau KW (1992) Retinal rods and cones have distinct G protein beta and gamma subunits. Proc Natl Acad Sci U S A 89:10882–10886
85. Fung BK, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc Natl Acad Sci U S A 78:152–156
86. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB (1996) The 2.0 A crystal structure of a heterotrimeric G protein. Nature 379:311–319
87. Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB (1996) Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. (See comments] [corrected] [published erratum appears in Nature 1996 Feb 29;379(6568):847). Nature 379:369–374
88. Sondek J, Lambright DG, Noel JP, Hamm HE, Sigler PB (1994) GTPase mechanism of G-proteins from the 1.7-A crystal structure of transducin alpha-GDP-AIF-4. (See comments). Nature 372:276–279
89. Calvert PD, Krasnoperova NV, Lyubarsky AL, Isayama T, Nicolo M, Kosaras B, Wong G, Gannon KS, Margolskee RF, Sidman RL et al (2000) Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha -subunit. Proc Natl Acad Sci U S A 97:13913–13918
90. Bornancin F, Pfister C, Chabre M (1989) The transitory complex between photoexcited rhodopsin and transducin. Reciprocal interaction between the retinal site in rhodopsin and the nucleotide site in transducin. Eur J Biochem 184:687–698
91. Jastrzebska B, Golczak M, Fotiadis D, Engel A, Palczewski K (2009) Isolation and functional characterization of a stable complex between photoactivated rhodopsin and the G protein, transducin. FASEB J 23:371–381
92. Heck M, Hofmann KP (2001) Maximal rate and nucleotide dependence of rhodopsin-catalyzed transducin activation: initial rate analysis based on a double displacement mechanism. J Biol Chem 276:10000–10009
93. Leskov IB, Klenchin VA, Handy JW, Whitlock GG, Govardovskii VI, Bownds MD, Lamb TD, Pugh EN Jr, Arshavsky VY (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. Neuron 27:525–537
94. Yee R, Liebman PA (1978) Light-activated phosphodiesterase of the rod outer segment. Kinetics and parameters of activation and deactivation. J Biol Chem 253:8902–8909
95. Burns ME, Pugh EN Jr (2010) Lessons from photoreceptors: turning off g-protein signaling in living cells. Physiology (Bethesda) 25:72–84.
96. Krispel CM, Chen D, Melling N, Chen YJ, Martemyanov KA, Quillinan N, Arshavsky VY, Wensel TG, Chen CK, Burns ME (2006) RGS expression rate-limits recovery of rod photoresponses. Neuron 51:409–416
97. Kleuss C, Raw AS, Lee E, Sprang SR, Gilman AG (1994) Mechanism of GTP hydrolysis by G-protein alpha subunits. Proc Natl Acad Sci U S A 91:9828–9831

98. Kjeldgaard M, Nyborg J, Clark BF (1996) The GTPbinding motif: variations on a theme. FASEB J 10:1347–1368
99. Chung KY, Rasmussen SG, Liu T, Li S, DeVree BT, Chae PS, Calinski D, Kobilka BK, Woods VL Jr, Sunahara RK (2011) Conformational changes in the G protein Gs induced by the beta2 adrenergic receptor. Nature 477:611–615
100. Kisselev OG, Park JH, Choe H-W, Ernst OP (2011) Signal transfer from receptor to G protein: the rhodopsin-transducin model. In Giraldo J, Pin JG (eds) G Protein-coupled receptors: from structure to function. Royal Society of Chemistry, London, pp 54–74
101. Papermaster DS, Dreyer WJ (1974) Rhodopsin content in the outer segment membranes of bovine and frog retinal rods. Biochemistry 13:2438–2444
102. Fung BK (1983) Characterization of transducin from bovine retinal rod outer segments. I. Separation and reconstitution of the subunits. J Biol Chem 258:10495–10502
103. Kühn H (1980) Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. Nature 283:587–589
104. Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9:60–71
105. Kokame K, Fukada Y, Yoshizawa T, Takao T, Shimonishi Y (1992) Lipid modification at the N terminus of photoreceptor G-protein alpha-subunit. Nature 359:749–752
106. Fukada Y, Takao T, Ohguro H, Yoshizawa T, Akino T, Shimonishi Y (1990) Farnesylated gamma-subunit of photoreceptor G protein indispensable for GTP-binding. Nature 346:658–660
107. Wedegaertner PB, Wilson PT, Bourne HR (1995) Lipid modifications of trimeric G proteins. J Biol Chem 270:503–506
108. Kassai H, Aiba A, Nakao K, Nakamura K, Katsuki M, Xiong WH, Yau KW, Imai H, Shichida Y, Satomi Y et al (2005) Farnesylation of retinal transducin underlies its translocation during light adaptation. Neuron 47:529–539
109. Simonds WF, Butrynski JE, Gautam N, Unson CG, Spiegel AM (1991) G-protein beta gamma dimers. Membrane targeting requires subunit coexpression and intact gamma C-A-A-X domain. J Biol Chem 266:5363–5366
110. Shinozawa T, Uchida S, Martin E, Cafiso D, Hubbell W, Bitensky M (1980) Additional component required for activity and reconstitution of light-activated vertebrate photoreceptor GTPase. Proc Natl Acad Sci U S A 77:1408–1411
111. Kolesnikov AV, Rikimaru L, Hennig AK, Lukasiewicz PD, Fliesler SJ, Govardovskii VI, Kefalov VJ, Kisselev OG (2011) G-protein betagamma-complex is crucial for efficient signal amplification in vision. J Neurosci 31:8067–8077
112. Lobanova ES, Finkelstein S, Herrmann R, Chen YM, Kessler C, Michaud NA, Trieu LH, Strissel KJ, Burns ME, Arshavsky VY (2008) Transducin gamma-subunit sets expression levels of alpha- and beta-subunits and is crucial for rod viability. J Neurosci 28:3510–3520
113. Nikonov SS, Lyubarsky A, Fina ME, Nikonova ES, Sengupta A, Chinniah C, Ding XQ, Smith RG, Pugh EN Jr, Vardi N et al (2013) Cones respond to light in the absence of transducin beta subunit. J Neurosci 33:5182–5194
114. Jastrzebska B, Tsybovsky Y, Palczewski K (2010) Complexes between photoactivated rhodopsin and transducin: progress and questions. Biochem J 428:1–10
115. Thaker TM, Kaya AI, Preininger AM, Hamm HE, Iverson TM (2012) Allosteric mechanisms of G protein-coupled receptor signaling: a structural perspective. Methods Mol Biol 796:133–174
116. Rasmussen SG, Devree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D et al (2011) Crystal structure of the beta(2) adrenergic receptor-Gs protein complex. Nature 19:549–555
117. Palczewski K (2010) Oligomeric forms of G protein-coupled receptors (GPCRs). Trends Biochem Sci 35:595–600
118. Slep KC, Kercher MA, He W, Cowan CW, Wensel TG, Sigler PB (2001) Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 A. Nature 409:1071–1077

119. Lomonosova E, Kolesnikov AV, Kefalov VJ, Kisselev OG (2012) Signaling states of rhodopsin in rod disk membranes lacking transducin betagamma-complex. Invest Ophthalmol Vis Sci 53:1225–1233
120. Bockaert J, Deterre P, Pfister C, Guillon G, Chabre M (1985) Inhibition of hormonally regulated adenylate cyclase by the beta gamma subunit of transducin. EMBO J 4:1413–1417
121. Tachibanaki S, Tsushima S, Kawamura S (2001) Low amplification and fast visual pigment phosphorylation as mechanisms characterizing cone photoresponses. Proc Natl Acad Sci U S A 98:14044–14049
122. Starace DM, Knox BE (1997) Activation of transducin by a Xenopus short wavelength visual pigment. J Biol Chem 272:1095–1100
123. Deng WT, Sakurai K, Liu J, Dinculescu A, Li J, Pang J, Min SH, Chiodo VA, Boye SL, Chang B et al (2009) Functional interchangeability of rod and cone transducin alpha-subunits. Proc Natl Acad Sci U S A 106:17681–17686
124. Chen CK, Woodruff ML, Chen FS, Shim H, Cilluffo MC, Fain GL (2010) Replacing the rod with the cone transducin subunit decreases sensitivity and accelerates response decay. J Physiol 588:3231–3241
125. Mao W, Miyagishima KJ, Yao Y, Soreghan B, Sampath AP, Chen J (2013) Functional comparison of rod and cone Galpha(t) on the regulation of light sensitivity. J Biol Chem 288:5257–5267
126. Gopalakrishna KN, Boyd KK, Artemyev NO (2012) Comparative analysis of cone and rod transducins using chimeric Galpha subunits. Biochemistry 51:1617–1624
127. Kisselev OG, Kolesnikov AV, Lobysheva EL, Kefalov VJ (2013) Replacement of rod-specific transducin gamma subunit in mouse rod photoreceptors. FASEB, Biology and Chemistry of Vision, Steamboat Springs, CO, June 9–June 14, 2013
128. Pronin AN, Gautam N (1992) Interaction between G-protein beta and gamma subunit types is selective. Proc Natl Acad Sci U S A 89:6220–6224
129. Humrich J, Bermel C, Bunemann M, Harmark L, Frost R, Quitterer U, Lohse MJ (2005) Phosducin-like protein regulates G-protein betagamma folding by interaction with tailless complex polypeptide-1alpha: dephosphorylation or splicing of PhLP turns the switch toward regulation of Gbetagamma folding. J Biol Chem 280:20042–20050
130. Lukov GL, Hu T, McLaughlin JN, Hamm HE, Willardson BM (2005) Phosducin-like protein acts as a molecular chaperone for G protein betagamma dimer assembly. EMBO J 24:1965–1975
131. Lukov GL, Baker CM, Ludtke PJ, Hu T, Carter MD, Hackett RA, Thulin CD, Willardson BM (2006) Mechanism of assembly of G protein betagamma subunits by protein kinase CK2-phosphorylated phosducin-like protein and the cytosolic chaperonin complex. J Biol Chem 281:22261–22274
132. Howlett AC, Gray AJ, Hunter JM, Willardson BM (2009) Role of molecular chaperones in G protein beta5/regulator of G protein signaling dimer assembly and G protein betagamma dimer specificity. J Biol Chem 284:16386–16399
133. Schmidt CJ, Neer EJ (1991) *In vitro* synthesis of G protein beta gamma dimers. J Biol Chem 266:4538–4544
134. Higgins JB, Casey PJ (1994) *In vitro* processing of recombinant G protein gamma subunits. Requirements for assembly of an active beta gamma complex. J Biol Chem 269:9067–9073
135. Pronin AN, Gautam N (1993) Proper processing of a G protein gamma subunit depends on complex formation with a beta subunit. FEBS Lett 328:89–93
136. Wells CA, Dingus J, Hildebrandt JD (2006) Role of the chaperonin CCT/TRiC complex in G protein betagamma-dimer assembly. J Biol Chem 281:20221–20232
137. Llorca O, Martin-Benito J, Grantham J, Ritco-Vonsovici M, Willison KR, Carrascosa JL, Valpuesta JM (2001) The 'sequential allosteric ring' mechanism in the eukaryotic chaperonin-assisted folding of actin and tubulin. EMBO J 20:4065–4075
138. Valpuesta JM, Martin-Benito J, Gomez-Puertas P, Carrascosa JL, Willison KR (2002) Structure and function of a protein folding machine: the eukaryotic cytosolic chaperonin CCT. FEBS Lett 529:11–16

139. Cong Y, Baker ML, Jakana J, Woolford D, Miller EJ, Reissmann S, Kumar RN, Redding-Johanson AM, Batth TS, Mukhopadhyay A et al (2010) 4.0-A resolution cryo-EM structure of the mammalian chaperonin TRiC/CCT reveals its unique subunit arrangement. Proc Natl Acad Sci U S A 107:4967–4972
140. Dekker C, Roe SM, McCormack EA, Beuron F, Pearl LH, Willison KR (2011) The crystal structure of yeast CCT reveals intrinsic asymmetry of eukaryotic cytosolic chaperonins. EMBO J 30:3078–3090
141. Meyer AS, Gillespie JR, Walther D, Millet IS, Doniach S, Frydman J (2003) Closing the folding chamber of the eukaryotic chaperonin requires the transition state of ATP hydrolysis. Cell 113:369–381
142. Munoz IG, Yebenes H, Zhou M, Mesa P, Serna M, Park AY, Bragado-Nilsson E, Beloso A, de Carcer G, Malumbres M et al (2011) Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. Nat Struct Mol Biol 18:14–19
143. Kwok MC, Holopainen JM, Molday LL, Foster LJ, Molday RS (2008) Proteomics of photoreceptor outer segments identifies a subset of SNARE and Rab proteins implicated in membrane vesicle trafficking and fusion. Mol Cell Proteomics 7:1053–1066
144. Song H, Sokolov M (2009) Analysis of protein expression and compartmentalization in retinal neurons using serial tangential sectioning of the retina. J Proteome Res 8:346–351
145. Reidel B, Thompson JW, Farsiu S, Moseley MA, Skiba NP, Arshavsky VY (2011) Proteomic profiling of a layered tissue reveals unique glycolytic specializations of photoreceptor cells. Mol Cell Proteomics 10:M110.002469
146. Lobanova ES, Finkelstein S, Skiba NP, Arshavsky VY (2013) Proteasome overload is a common stress factor in multiple forms of inherited retinal degeneration. Proc Natl Acad Sci U S A 110:9986–9991
147. Posokhova E, Song H, Belcastro M, Higgins L, Bigley LR, Michaud NA, Martemyanov KA, Sokolov M (2011) Disruption of the chaperonin containing TCP-1 function affects protein networks essential for rod outer segment morphogenesis and survival. Mol Cell Proteomics 10:M110.000570
148. Obin M, Lee BY, Meinke G, Bohm A, Lee RH, Gaudet R, Hopp JA, Arshavsky VY, Willardson BM, Taylor A (2002) Ubiquitylation of the transducin betagamma subunit complex. Regulation by phosducin. J Biol Chem 277:44566–44575
149. Kubota S, Kubota H, Nagata K (2006) Cytosolic chaperonin protects folding intermediates of Gbeta from aggregation by recognizing hydrophobic beta-strands. Proc Natl Acad Sci U S A 103:8360–8365
150. Lai CW, Kolesnikov AV, Frederick JM, Blake DR, Jiang L, Stewart JS, Chen CK, Barrow JR, Baehr W, Kefalov VJ et al (2013) Phosducin-like protein 1 is essential for G-protein assembly and signaling in retinal rod photoreceptors. J Neurosci 33:7941–7951
151. Miles MF, Barhite S, Sganga M, Elliott M (1993) Phosducin-like protein: an ethanol-responsive potential modulator of guanine nucleotide-binding protein function. Proc Natl Acad Sci U S A 90:10831–10835
152. Thulin CD, Howes K, Driscoll CD, Savage JR, Rand TA, Baehr W, Willardson BM (1999) The immunolocalization and divergent roles of phosducin and phosducin-like protein in the retina. Mol Vis 5:40
153. McLaughlin JN, Thulin CD, Hart SJ, Resing KA, Ahn NG, Willardson BM (2002) Regulatory interaction of phosducin-like protein with the cytosolic chaperonin complex. Proc Natl Acad Sci U S A 99:7962–7967
154. Martin-Benito J, Bertrand S, Hu T, Ludtke PJ, McLaughlin JN, Willardson BM, Carrascosa JL, Valpuesta JM (2004) Structure of the complex between the cytosolic chaperonin CCT and phosducin-like protein. Proc Natl Acad Sci U S A 101:17410–17415
155. Humrich J, Bermel C, Grubel T, Quitterer U, Lohse MJ (2003) Regulation of phosducin-like protein by casein kinase 2 and N-terminal splicing. J Biol Chem 278:4474–4481
156. Olsten ME, Weber JE, Litchfield DW (2005) CK2 interacting proteins: emerging paradigms for CK2 regulation? Mol Cell Biochem 274:115–124

157. Gao X, Sinha S, Belcastro M, Woodard C, Ramamurthy V, Stoilov P, Sokolov M (2013) Splice isoforms of Phosducin-like protein control the expression of heterotrimeric G proteins. J Biol Chem 288:25760–25768
158. Yam AY, Xia Y, Lin HT, Burlingame A, Gerstein M, Frydman J (2008) Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat Struct Mol Biol 15:1255–1262
159. Kabir MA, Uddin W, Narayanan A, Reddy PK, Jairajpuri MA, Sherman F, Ahmad Z (2011) Functional subunits of eukaryotic chaperonin CCT/TRiC in protein folding. J Amino Acids 2011:843206
160. Craft CM, Xu J, Slepak VZ, Zhan-Poe X, Zhu X, Brown B, Lolley RN (1998) PhLPs and PhLOPs in the phosducin family of G beta gamma binding proteins. Biochemistry 37:15758–15772
161. Dupre DJ, Robitaille M, Richer M, Ethier N, Mamarbachi AM, Hebert TE (2007) Dopamine receptor-interacting protein 78 acts as a molecular chaperone for Ggamma subunits before assembly with Gbeta. J Biol Chem 282:13703–13715
162. Sha B, Lee S, Cyr DM (2000) The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. Structure 8:799–807
163. Shi YY, Hong XG, Wang CC (2005) The C-terminal (331–376) sequence of *Escherichia coli* DnaJ is essential for dimerization and chaperone activity: a small angle X-ray scattering study in solution. J Biol Chem 280:22761–22768
164. Gaudet R, Bohm A, Sigler PB (1996) Crystal structure at 2.4 angstroms resolution of the complex of transducin betagamma and its regulator, phosducin. Cell 87:577–588
165. Cohen AI, Todd RD, Harmon S, O'Malley KL (1992) Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. Proc Natl Acad Sci U S A 89:12093–12097
166. Vuvan T, Geffard M, Denis P, Simon A, Nguyen-Legros J (1993) Radioimmunoligand characterization and immunohistochemical localization of dopamine D2 receptors on rods in the rat retina. Brain Res 614:57–64
167. Nguyen-Legros J, Chanut E, Versaux-Botteri C, Simon A, Trouvin JH (1996) Dopamine inhibits melatonin synthesis in photoreceptor cells through a D2-like receptor subtype in the rat retina: biochemical and histochemical evidence. J Neurochem 67:2514–2520
168. Klitten LL, Rath MF, Coon SL, Kim JS, Klein DC, Moller M (2008) Localization and regulation of dopamine receptor D4 expression in the adult and developing rat retina. Exp Eye Res 87:471–477
169. Schmitz F, Tabares L, Khimich D, Strenzke N, de la Villa-Polo P, Castellano-Munoz M, Bulankina A, Moser T, Fernandez-Chacon R, Sudhof TC (2006) CSPalpha-deficiency causes massive and rapid photoreceptor degeneration. Proc Natl Acad Sci U S A 103:2926–2931
170. Natochin M, Campbell TN, Barren B, Miller LC, Hameed S, Artemyev NO, Braun JE (2005) Characterization of the G alpha(s) regulator cysteine string protein. J Biol Chem 280:30236–30241
171. Lai RK, Perez-Sala D, Canada FJ, Rando RR (1990) The gamma subunit of transducin is farnesylated. Proc Natl Acad Sci U S A 87:7673–7677
172. Otto JC, Kim E, Young SG, Casey PJ (1999) Cloning and characterization of a mammalian prenyl protein-specific protease. J Biol Chem 274:8379–8382
173. Christiansen JR, Kolandaivelu S, Bergo MO, Ramamurthy V (2011) RAS-converting enzyme 1-mediated endoproteolysis is required for trafficking of rod phosphodiesterase 6 to photoreceptor outer segments. Proc Natl Acad Sci U S A 108:8862–8866
174. Ohguro H, Fukada Y, Takao T, Shimonishi Y, Yoshizawa T, Akino T (1991) Carboxyl methylation and farnesylation of transducin gamma-subunit synergistically enhance its coupling with metarhodopsin II. EMBO J 10:3669–3674
175. Bergo MO, Leung GK, Ambroziak P, Otto JC, Casey PJ, Young SG (2000) Targeted inactivation of the isoprenylcysteine carboxyl methyltransferase gene causes mislocalization of K-Ras in mammalian cells. J Biol Chem 275:17605–17610

176. Fukada Y, Matsuda T, Kokame K, Takao T, Shimonishi Y, Akino T, Yoshizawa T (1994) Effects of carboxyl methylation of photoreceptor G protein gamma-subunit in visual transduction. J Biol Chem 269:5163–5170
177. Marrari Y, Crouthamel M, Irannejad R, Wedegaertner PB (2007) Assembly and trafficking of heterotrimeric G proteins. Biochemistry 46:7665–7677
178. Bigay J, Faurobert E, Franco M, Chabre M (1994) Roles of lipid modifications of transducin subunits in their GDP-dependent association and membrane binding. Biochemistry 33:14081–14090
179. Katadae M, Hagiwara K, Wada A, Ito M, Umeda M, Casey PJ, Fukada Y (2008) Interacting targets of the farnesyl of transducin gamma-subunit. Biochemistry 47:8424–8433
180. Loew A, Ho YK, Blundell T, Bax B (1998) Phosducin induces a structural change in transducin beta gamma. Structure 6:1007–1019
181. Neubert TA, Johnson RS, Hurley JB, Walsh KA (1992) The rod transducin alpha subunit amino terminus is heterogeneously fatty acylated. J Biol Chem 267:18274–18277
182. Kerov V, Rubin WW, Natochin M, Melling NA, Burns ME, Artemyev NO (2007) N-terminal fatty acylation of transducin profoundly influences its localization and the kinetics of photoresponse in rods. J Neurosci 27:10270–10277
183. Farr GW, Scharl EC, Schumacher RJ, Sondek S, Horwich AL (1997) Chaperonin-mediated folding in the eukaryotic cytosol proceeds through rounds of release of native and nonnative forms. Cell 89:927–937
184. Kerr DS, Von Dannecker LE, Davalos M, Michaloski JS, Malnic B (2008) Ric-8B interacts with G alpha olf and G gamma 13 and co-localizes with G alpha olf, G beta 1 and G gamma 13 in the cilia of olfactory sensory neurons. Mol Cell Neurosci 38:341–348
185. Nagai Y, Nishimura A, Tago K, Mizuno N, Itoh H (2010) Ric-8B stabilizes the alpha subunit of stimulatory G protein by inhibiting its ubiquitination. J Biol Chem 285:11114–11120
186. Gabay M, Pinter ME, Wright FA, Chan P, Murphy AJ, Valenzuela DM, Yancopoulos GD, Tall GG (2011) Ric-8 proteins are molecular chaperones that direct nascent G protein alpha subunit membrane association. Sci Signal 4:ra79
187. Chan P, Thomas CJ, Sprang SR, Tall GG (2013) Molecular chaperoning function of Ric-8 is to fold nascent heterotrimeric G protein alpha subunits. Proc Natl Acad Sci U S A 110:3794–3799
188. Vaiskunaite R, Kozasa T, Voyno-Yasenetskaya TA (2001) Interaction between the G alpha subunit of heterotrimeric G(12) protein and Hsp90 is required for G alpha(12) signaling. J Biol Chem 276:46088–46093
189. Waheed AA, Jones TL (2002) Hsp90 interactions and acylation target the G protein Galpha 12 but not Galpha 13 to lipid rafts. J Biol Chem 277:32409–32412
190. Dean DO, Kent CR, Tytell M (1999) Constitutive and inducible heat shock protein 70 immunoreactivity in the normal rat eye. Invest Ophthalmol Vis Sci 40:2952–2962
191. Helmlinger D, Bonnet J, Mandel JL, Trottier Y, Devys D (2004) Hsp70 and Hsp40 chaperones do not modulate retinal phenotype in SCA7 mice. J Biol Chem 279:55969–55977
192. Zhang H, Huang W, Zhang H, Zhu X, Craft CM, Baehr W, Chen CK (2003) Light-dependent redistribution of visual arrestins and transducin subunits in mice with defective phototransduction. Mol Vis 9:231–237
193. Zhang H, Li S, Doan T, Rieke F, Detwiler PB, Frederick JM, Baehr W (2007) Deletion of PrBP/delta impedes transport of GRK1 and PDE6 catalytic subunits to photoreceptor outer segments. Proc Natl Acad Sci U S A 104:8857–8862
194. Zhang H, Constantine R, Vorobiev S, Chen Y, Seetharaman J, Huang YJ, Xiao R, Montelione GT, Gerstner CD, Davis MW et al (2011) UNC119 is required for G protein trafficking in sensory neurons. Nat Neurosci 14:874–880
195. Gopalakrishna KN, Doddapuneni K, Boyd KK, Masuho I, Martemyanov KA, Artemyev NO (2011) Interaction of transducin with uncoordinated 119 protein (UNC119): implications for the model of transducin trafficking in rod photoreceptors. J Biol Chem 286:28954–28962

196. Schwarz N, Hardcastle AJ, Cheetham ME (2012) Arl3 and RP2 mediated assembly and traffic of membrane associated cilia proteins. Vision Res 75:2–4
197. Schwarz N, Novoselova TV, Wait R, Hardcastle AJ, Cheetham ME (2012) The X-linked retinitis pigmentosa protein RP2 facilitates G protein traffic. Hum Mol Genet 21:863–873
198. Sinha S, Majumder A, Belcastro M, Sokolov M, Artemyev NO (2013) Expression and subcellular distribution of UNC119a, a protein partner of transducin alpha subunit in rod photoreceptors. Cell Signal 25:341–348
199. Sokolov M, Strissel KJ, Leskov IB, Michaud NA, Govardovskii VI, Arshavsky VY (2004) Phosducin facilitates light-driven transducin translocation in rod photoreceptors. Evidence from the phosducin knockout mouse. J Biol Chem 279:19149–19156

Chapter 4
G-Protein–Effector Coupling in the Vertebrate Phototransduction Cascade

Nikolai O. Artemyev

Abstract For many years, the rod phototransduction cascade has served as a valuable model to study the mechanisms of cellular signaling by G-protein-coupled receptors (GPCRs). Our understanding of the key steps of signal transfer from GPCRs to G proteins and further downstream to G protein effectors has been greatly advanced through extensive biochemical studies in rod photoreceptors. This review focuses on the coupling of the visual G protein transducin to its classical effector phosphodiesterase 6. A new level of mechanistic insight has been achieved from the atomic structures of the signaling molecules. Recent studies on light-dependent translocation of transducin in rods raise a possibility for noncanonical transducin signaling and partners in the photoreceptor synaptic terminal.

Introduction

The visual transduction cascade in vertebrate rod photoreceptors has served for many years as a paradigm for G-protein signaling. Phototransduction takes place in a specialized ciliary compartment of photoreceptor cells called the outer segment (OS). Following absorption of a photon of light, photoexcited rhodopsin (R*) stimulates guanosine triphosphate (GTP)–guanosine diphosphate (GDP) exchange on the retinal G protein, transducin, resulting in dissociation of $G\alpha_t$GTP from $G\beta_1\gamma_1$ and R*. $G\alpha_t$ in the GTP-bound conformation activates the effector enzyme cyclic guanosine monophosphate (cGMP) phosphodiesterase 6 (PDE6) by displacing the inhibitory γ subunits (Pγ) from the PDE6 catalytic core (PDE6AB). cGMP hydrolysis by active PDE6 produces closure of cGMP-gated channels in the plasma

NOA research is supported by NIH grants EY10843 and EY12682

N. O. Artemyev (✉)
Department of Molecular Physiology and Biophysics, Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA 52242, USA
e-mail: nikolai-artemyev@uiowa.edu

© Springer Science+Business Media New York 2014
K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_4

membrane [1–4]. The lifetime of the activated complex between $G\alpha_t$GTP and PDE6 is controlled by the GTPase activity of $G\alpha_t$. Hydrolysis of GTP transforms the GTP-bound conformation of $G\alpha_t$ into the inactive GDP-bound state and allows for re-inhibition of PDE6AB by Pγ. A photoreceptor-specific member of the regulators of G-protein signaling (RGS) family, RGS9-1, in complex with $G\beta_{5L}$ acts as a GTPase-activating protein (GAP) for $G\alpha_t$, and thus is a major regulator of the turn-off kinetics of the visual signal [4–9]. The Pγ subunits potentiate the GAP action of RGS9-1 [10, 11] by increasing the affinity between $G\alpha_t$ and RGS9-1/Gβ5L [12]. Here, I review general aspects of the G-protein–effector coupling in photoreceptor cells. Our current understanding of this step of phototransduction was built initially upon extensive biochemical studies, and subsequently it has been greatly advanced by the atomic structures of the key signaling molecules. Lastly, a novel aspect of transducin signaling in rods is discussed. A long-recognized phenomenon of light-induced translocation of rod transducin from the OS to the inner segment (IS)/synaptic terminal is thought to contribute to light adaptation and neuroprotection of rods [13–17]. New findings suggest that transducin translocation in rods enhances signaling to rod bipolar cells, apparently by interacting with as yet undetermined partners or effectors in the synaptic terminal of photoreceptor cells [18].

HoloPDE6: Inhibition of the Catalytic Subunits by the Inhibitory Pγ Subunits

Eleven families of phosphodiesterases (PDEs) of cyclic nucleotides have been identified in mammalian tissues based on primary sequence, substrate selectivity, and regulation [19, 20]. Photoreceptor rod and cone PDEs comprise the PDE6 family. In various species, rod photoreceptor PDE is composed of two large homologous catalytic α (PDE6A) and β subunits (PDE6B) of ~90–100 kDa and two copies of an inhibitory Pγ subunit of ~10 kDa [21–27]. The degree of homology between PDE6A and PDE6B subunits is 70–75 % [27]. Cone PDE is composed of two identical PDE6C subunits which share >60 % homology with PDE6A and PDE6B [28]. A cone-specific inhibitory Pγ subunit is highly homologous to the rod Pγ [29]. Each PDE6 subunit in the catalytic dimer is composed of three structural domains: two N-terminal regulatory GAF domains (GAFa and GAFb) and the conserved C-terminal PDE catalytic domain of about 300 amino acid (aa) residues [30] (Fig. 4.1a). The GAFa domains in PDE6 serve as the site for noncatalytic binding of cGMP [31, 32]. The high-resolution structure of PDE6 is not available. However, the atomic structure of the individual chicken cone PDE6 GAFa domain has been solved [32], and models of the PDE6 catalytic domains have been generated based on the structures of the related catalytic domains of PDE5 and PDE5/6 chimera [33–35].

A general map of the Pγ–PDE6 interactions has been defined in biochemical studies. Two regions of Pγ are primarily involved in the interaction with the PDE6 catalytic subunits, the proline-rich polycationic region (approximately residues 20–45 of rod Pγ) and the Pγ C-terminus [36–43] (Fig. 4.1a). The polycationic

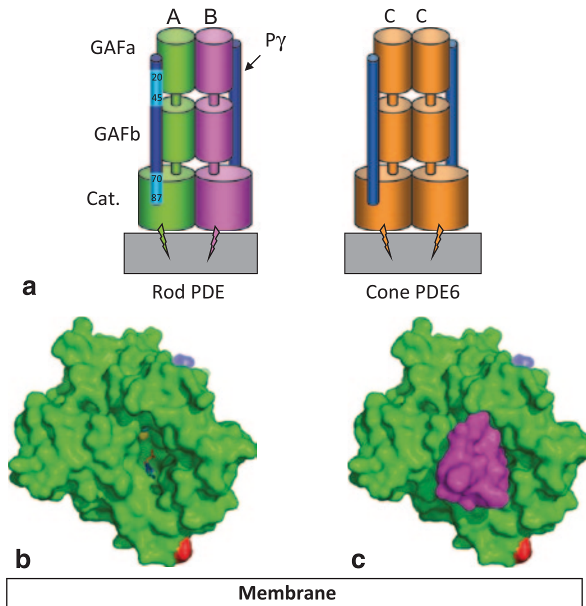

Fig. 4.1 **a** Cartoon depicting the subunit composition and domain structure of rod and cone phosphodiesterase 6 (PDE6). The inhibitory γ subunits (Pγ) region ~20–45 interacts with the *GAFa* domain of PDE6, whereas the C-terminal residues ~70–87 bind the *PDE6* catalytic domain. **b** Surface representation of the PDE5/6 catalytic domain without bound Pγ70–87 showing the catalytic cavity. The PDE catalytic site contains zinc (*yellow sphere*) and magnesium (*cyan sphere*) and the IBMX inhibitor molecule (*sticks*) (PDB ID: 3JWR) [35]. **c** The PDE5/6 catalytic domain with bound Pγ70–87 (magenta) showing how the Pγ C-terminal peptide occludes the catalytic site (PDB ID: 3JWR) [35]. The N-terminal residue in the structure of PDE5/6cd is shown in *blue,* and the C-terminal residue is shown in *red* (**b, c**). The C-termini of PDE6 catalytic subunits are isoprenylated, suggesting a probable membrane orientation

region of Pγ binds to the PDE6 GAFa domain and contributes significantly to the overall affinity of Pγ for PDE6 catalytic subunits. The C-terminus of Pγ constitutes the key inhibitory domain. It interacts with the PDE6 catalytic domain and occludes the active site [39, 40]. Free Pγ appears to be an intrinsically disordered protein with elements of transient secondary and tertiary structure [44]. Upon binding to PDE6AB, Pγ apparently adopts an extended conformation [43]. This allows Pγ to bind simultaneously to the PDE6 GAFa domain and the catalytic site separated by a distance of more than 50–60 Å [45]. The noncatalytic cGMP bound at the PDE6 GAFa domain enhances affinity of Pγ for the PDE6 catalytic subunits. Reciprocally, Pγ increases the binding of noncatalytic cGMP [41, 46]. The interface between the polycationic region of Pγ and the GAFa domain is yet to be fully elucidated. Nonetheless, cross-linking studies, mutational analyses of the PDE6C GAFa domain, and the structure of the PDE6C GAFa revealed a conserved surface consisting of negatively charged and hydrophobic residues that participate in binding of the Pγ polycationic region [31, 32, 42]. This surface is in close proximity to the opening of

the binding pocket for noncatalytic cGMP [31, 32]. Thus, the positive cooperativity between noncatalytic cGMP and Pγ arises in all probability from direct mutual stabilization of the binding sites. The functional significance of this cooperative regulation for phototransduction, however, remains elusive [46].

The Structure of the PDE-Bound Pγ C-Terminus Reveals Molecular Details of PDE6 Inhibition

The atomic structure of the chimeric PDE5/PDE6 catalytic domain (PDE5/6c) bound to the inhibitory peptide of Pγ, Pγ70–87, provided the first structural insights into the mechanism of PDE6 inhibition by Pγ [35] (Figs. 4.1b, c and 4.2). In the structure, residues Pγ75–83 form an α-helix and residues Pγ 84–87 form a cap near the active site [35]. The interactions between Pγ70–87 and PDE5/6cd are a combination of hydrophobic, electrostatic, and Van der Waals interactions with hydrophobic interactions providing a major contribution. The PDE5/6-Pγ70–87 structure is consistent with the role of the PDE6 M-loop/α-helix 15 region containing two key residues, Met and Phe (M804 and F823 in PDE5/6cd; Fig. 4.2). These residues have been implicated in the inhibitory interaction with Pγ in biochemical studies [47, 48]. Moreover, the roles of many putative PDE6 contact residues of Pγ suggested previously [37, 49] have been supported and elaborated by the structure [35]. M-loop residues I802 and M804 interact with Pγ residues A82, Q83, Y84, and G85, and α-helix 15 residues F820, F823, and V824 make contacts with E80, L81, Q83, and Y84 of Pγ [35]. PDE5/6cd I802, P803, M804, and L816 form a barrier, thus terminating the α-helix of Pγ and inducing the C-terminal cap, which is also stabilized by intramolecular Pγ interactions involving hydrophobic residues [35]. As a result, the Pγ peptide completely blocks the active site of PDE5/6cd by occluding the opening of the catalytic cavity (Fig. 4.1b, c).

The Membrane-Bound Complex Between $G\alpha_t$GTP and PDE6

Following the R*-dependent activation of the heterotrimeric transducin and dissociation of $G\beta_1\gamma_1$, $G\alpha_t$GTP diffuses along the surface of the disc membrane until it encounters holoPDE6. Although a single N-acyl lipid modification of $G\alpha_t$ does not provide for a strong membrane-binding affinity, it is unclear if $G\alpha_t$GTP significantly dissociates from the membrane prior to interaction with PDE6 [50–52]. This interaction leads to the formation of an active membrane-bound complex, $G\alpha_t$/Pγ-PDE6AB-Pγ/$G\alpha_t$ [53, 54]. The membrane attachment of the complex is secured by the N-acyl anchor of $G\alpha_t$ as well as by isoprenyl C-terminal lipids of PDE6AB [55]. The key binding sites for $G\alpha_t$GTP on holoPDE6 are presented by the Pγ subunits [1, 3, 56–59], although additional contacts of $G\alpha_t$ with PDE6AB have been

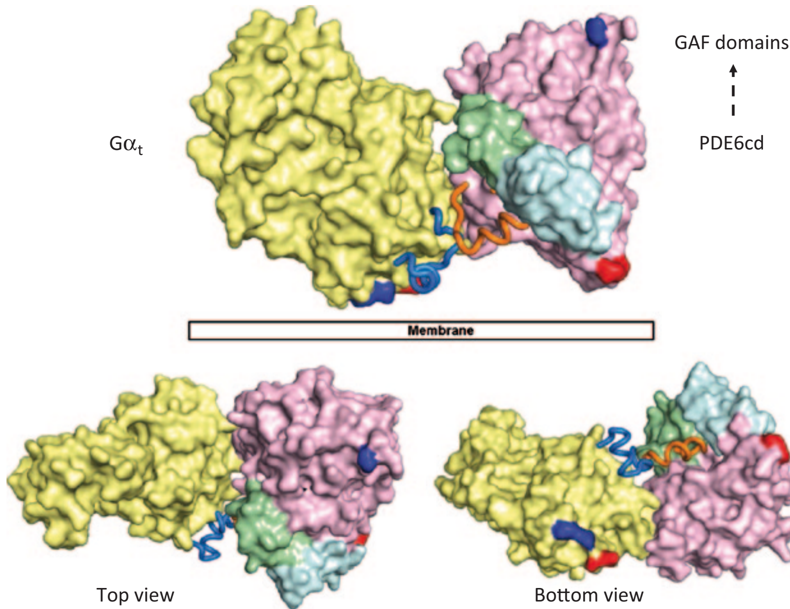

Fig. 4.2 A model of PDE6 activation by transducin. The proposed model [35] is based on the structure of the PDE5/6cd bound with Pγ70–87 (PDB ID: 3JWR) [35] and the Pγ C-terminal fragment bound to the transition-state complex of $G\alpha_t$ (PDB ID: 1FQJ) [59]. In holoPDE6, activity of the PDE6 catalytic domain (*light pink*; the Pγ-binding H- and M-loops are in *green* and *cyan,* respectively) is blocked by the Pγ C-terminus (*orange*). Activated $G\alpha_t$ (*yellow*) interacts with the PDE6-bound Pγ and induces a hinge-like rigid-body movement of Pγ 78–87 away from the PDE6 catalytic pocket causing Pγ 71–87 to adopt its $G\alpha_t$-bound conformation (*marine*; only residues Pγ 71–87 are shown from the Pγ 50–87 fragment in 1FQJ). The N-terminal residues in the structures of $G\alpha_t$ and PDE5/6cd are shown in *blue,* and the C-terminal residues are shown in *red.* The N-terminus of $G\alpha_t$ and the C-termini of PDE6 catalytic subunits are modified with lipids, suggesting probable membrane orientation of the signaling complex. In agreement, the C-terminus of $G\alpha_t$, a major R* recognition site, is facing the membrane. The extreme N-terminal PDE5/6cd residue is projected away from the membrane surface where it is expected to link with the PDE GAF domains. The *top view* indicates a potential cleft between $G\alpha_t$ and PDE5/6cd that may allow the Pγ24–46 region to extend towards the GAFa domain

suggested as well [60, 61]. The conventional model of rod PDE6 activation during phototransduction stipulates that $G\alpha_t$GTP binds equivalently to each Pγ in the complex, and the maximal PDE6 activation occurs with the displacement of both Pγ subunits by two $G\alpha_t$GTP molecules [3, 62]. Contrary to this view, several studies have shown that just one $G\alpha_t$ molecule can maximally activate the catalytic dimer [63–65]. The latter studies may imply that PDE6A-Pγ and PDE6B-Pγ have significantly different affinities for $G\alpha_t$GTP, and the binding of $G\alpha_t$ to the lower affinity site does not lead to PDE6 activation. Moreover, the lack of activation at the lower affinity site may indicate that one subunit, PDE6A or PDE6B, is catalytically deficient [66]. More recently, the analysis of chimeric PDE6 expressed in rods of transgenic *X. laevis* demonstrated the enzymatic equivalence of the PDE6A and

PDE6B catalytic subunits and supported the conventional model of PDE6 activation by transducin [67].

The activated $G\alpha_t$GTP/PDE6 complex is inactivated by the GAP complex that is also membrane bound. RGS9–1/$G\beta_{5L}$ is anchored to disc membranes via the transmembrane protein R9AP (RGS-9-1-anchor protein) [68]. This membrane attachment is shown to significantly enhance the GAP activity of RGS9-1/$G\beta_{5L}$ [69, 70]. Thus, both the activation and inactivation of PDE6 take place at the disc membrane surface, and the membrane interactions of the PDE6 signaling complex play a critical role in the kinetics and amplification of phototransduction [64, 71]. When activated, PDE6 is a nearly perfect enzyme with the catalytic efficiency k_{cat}/K_M exceeding $2 \times 10^8 M^{-1} s^{-1}$ [62]. This catalytic efficiency makes PDE6 an excellent "amplifier" in the phototransduction cascade.

Effector-Competent Conformation of $G\alpha_t$ and the Sites of Interaction with PDE6

The crystal structures of $G\alpha_t$GDP and $G\alpha_t$GTPγS coupled with the mutational analyses of transducin-α provided the first insights into the effector-interacting surface of the visual G protein. Three regions of $G\alpha_t$, called switch regions I, II, and III, assume significantly different conformations in the structure of $G\alpha_t$GTPγS compared to that of $G\alpha_t$GDP [72, 73]. The rearrangements linked to the interactions of the γ-phosphate of GTP lead to more ordered switch regions and prevent the binding of $G\beta_1\gamma_1$, which would substantially occlude the effector surface on $G\alpha_t$. Therefore, the switch regions represented potential contributors to the effector surface of $G\alpha_t$ [72, 73]. The switch II region of $G\alpha_t$, in particular, has emerged as a major contributor to the conformation-dependent interaction of $G\alpha_t$ with PDE6. The initial evidence was based on the inability of the $G\alpha_t$ switch II mutant, W207F, to activate PDE6 [56]. Further mutational analysis of the $G\alpha_t$ switch II region has revealed that in addition to W207, conserved R201, R204, and I208 are essential for the interaction with Pγ [58]. However, the switch II regions in Gα subunits from different families are highly conserved and alone cannot account for the G protein effector selectivity. In addition, biochemical studies on $G\alpha_t$/effector interactions indicated the presence of conformation-independent effector site(s). While $G\alpha_t$GDP is incapable of efficient activation of PDE6, it was shown to bind Pγ with ~20–30-fold lower affinity compared to $G\alpha_t$GTP [74, 75]. Moreover, analysis of the interaction of $G\alpha_t$/$G\alpha_i$ chimeras with Pγ suggested that the α3 helix-α3/β5 loop region in both $G\alpha_t$GDP and activated $G\alpha_t$GTP states participates in binding Pγ [57]. Subsequent studies revealed that the α3 helix also provides for the specificity of the $G\alpha_t$–Pγ interaction. Several residues in the α3 helix of $G\alpha_t$ were identified as responsible for the conformation-independent effector-specific interaction [58]. The role of the α3 helix becomes clearer in the context of the conformational changes of the switch II region upon activation of $G\alpha_t$. Bound GTP causes the switch II region, comprising the α2 helix and the α2/β4 loop, to stretch and rotate relative to the GDP-bound con-

formation, enabling the side chains of R201, R204, and W207 to form ordered interactions with residues E241, L245, and I249 from the α3 helix [72, 73]. Thereby, an effector-competent surface of $G\alpha_t$ is formed involving both the switch II and the α3 helix-α3/β5 loop regions.

The Interface Between $G\alpha_t$ and Pγ

Interestingly, the Pγ regions implicated in the interaction with $G\alpha_t$GTP are overlapping or adjacent to the Pγ site of interaction with the PDE6 catalytic subunits. The sites of Pγ for binding $G\alpha_t$GTP were localized to the polycationic region (Pγ-24–45) and the C-terminal region (Pγ-63–87) [76–78]. A cross-linking profiling of the Pγ interactions with PDE6AB and $G\alpha_t$GTP indicated the Pγ C-terminal region interacts preferentially with $G\alpha_t$GTP, whereas the central region of Pγ has a higher affinity for PDE6AB [77]. Furthermore, $G\alpha_t$GTP and PDE6AB co-immunoprecipitated in a Pγ-dependent manner, thus supporting simultaneous binding of the proteins to Pγ [77]. Thus, the complementary interactions of Pγ with PDE6AB and $G\alpha_t$GTP appear to facilitate the formation of the activated PDE6 complex with transducin-α. The weaker interaction of the Pγ C-terminus with PDE6AB may allow occasional openings of the catalytic pocket without full dissociation of Pγ. This may constitute a critical mechanism for the basal "dark" PDE6 activity. Basal PDE6 activity is essential to prevent elevation of free cGMP concentration that may cause retinal degeneration [79]. Basal PDE6 activity is also responsible for the continuous component of dark noise in rods [80]. On the other hand, the stronger interaction of $G\alpha_t$GTP with the Pγ C-terminus may provide for its effective displacement away from the catalytic pocket during the enzyme activation. In the activated complex, the central region of Pγ would keep Pγ tethered to PDE6AB. The specifics of the weaker $G\alpha_t$GTP interaction with the central region of Pγ remain obscure. In contrast, the structure of the Pγ C-terminal fragment Pγ-46-87 bound to the transition-state complex of $G\alpha_t$ offers important structural details into this part of the $G\alpha_t$–Pγ interface [59] (Fig. 4.2). The structure confirmed the switch II/α3-helix cleft as the key Pγ interaction region. The notion of the composite effector-activating surface composed of conformation-sensitive switch II residues as well as effector-specific residues in the α3-helix/α3-β5 loop was further supported [59]. The bound Pγ fragment forms three short α-helices with the middle helix interacting most intimately with switch II/α3 of $G\alpha_t$ [59]. This helix contains the critical W70 residue that is inserted into the switch II/α3 cleft of $G\alpha_t$ making multiple hydrophobic contacts with transducin residues [59]. The observed W70 contacts are in accord with the central role of W70 in the Pγ/ $G\alpha_t$ interaction and the GAP potentiation activity of Pγ demonstrated earlier [81]. Moreover, there is a small direct interface between Pγ and RGS9 in the ternary complex with $G\alpha_t$ [59], which is consistent with the Pγ role as the affinity adapter for the GAP complex [12] (Fig. 4.3).

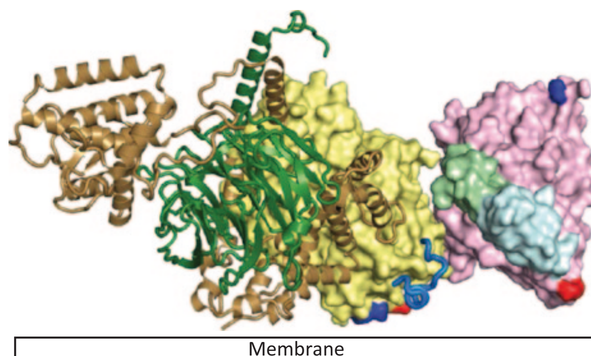

Fig. 4.3 A model of RGS9 GAP/Gα_t/PDE6 complex leading to inactivation of PDE6. A model for the activated Gα_t and PDE6 complex (as in Fig. 4.2) bound to RGS9/Gβ_5 was generated by superimposition of the RGS9 domains from the transition-state Gα_t structure (PDB ID: 1FQJ) [59] and the structure of the RGS9/Gβ_5 complex (PDB ID: 2PBI) [109]. RGS9 is shown in *brown*; Gβ_5 is *dark green.* The complex is tethered to the membrane by RGS-9-1-anchor protein R9AP (not shown)

The Structures of the PDE6- and Gα_t-Bound Pγ C-Terminus Illuminate the Mechanism of PDE6 Activation by Transducin

Comparison of the PDE5/6cd-bound conformation of the Pγ C-terminus [35] with that when bound to the transition-state complex of Gα_t [59] provides important insights into the mechanism of PDE6 activation by transducin. Several PDE6AB contact residues of Pγ, F73, L81, and I87 also interact with Gα_t [35, 59]. Thus, activation of PDE6 involves reorientation of these residues away from PDE6AB and sequestration by Gα_t. In addition, in the PDE5/6cd- and Gα_t-bound states, the Pγ C-terminal residues 78–87 assume similar conformations featuring α-helices and C-terminal caps [35, 59] (Fig. 4.2). Consequently, the ten C-terminal Cα atoms of Pγ in the two complexes are nearly superimposable. However, the Gα_t-bound conformation of residues 71–77 is different from that in the PDE5/6cd-bound state [35, 59]. A mechanism of PDE6 activation has been proposed whereby Gα_tGTP interaction with the PDE6-bound Pγ induces a conformational change within Pγ residues 71–77 and results in a hinge-like rigid-body movement of Pγ-78-87 away from the PDE6 catalytic pocket [35]. Two Gα_t-contact residues of Pγ, W70 and L76, while essential for the PDE6 activation by transducin, do not appear to contribute to the Pγ–PDE6AB binding [35, 49, 59, 81]. The Pγ-70-87/PDE5/6cd structure suggests that Pγ L76, and likely W70, are readily accessible for binding Gα_t. Thus, L76 and W70 may serve as anchors for the initial docking of Gα_tGTP. An additional docking site for Gα_t has been mapped to Pγ residues 52–54 [82]. Upon docking, Gα_t causes activating displacement of the Pγ C-terminus from PDE6 and sequesters Pγ residues F73, L81, and I87 released from the interface with PDE6 [35] (Fig. 4.2).

Light-Dependent Translocation of Transducin: New Partners and Functions in the Inner Compartments of Rods?

Exposure to bright light causes translocation of rod transducin from the OS to the IS and synaptic terminal [13–16]. Two common mechanisms for this translocation, diffusion and active transport, have been intensely investigated. At present, abundant biochemical evidence supports the diffusion model [83–85]. In a diffusion model, the light-induced dissociation of Gt subunits permits them to diffuse into the IS [16]. Light-induced translocation of transducin requires R* and does not take place in RPE65-knockout mice when regeneration of rhodopsin is blocked [86]. It occurs even in rods that are depleted of ATP [87, 88]. Phosducin facilitates translocation of Gt subunits through binding to $G\beta_1\gamma_1$ and sequestration of the $G\gamma_1$ farnesyl moiety [89]. An important feature of transducin translocation in rods is the triggering light intensity threshold, which is dependent on the presence and concentration of the RGS9 GAP complex [90, 91]. A prominent translocation of $G\alpha_t$ begins at light intensities causing ~5000 rhodopsin photoisomerizations per rod per second [91]. This threshold is lower in RGS9- and R9AP-knockout mice, and is higher in mice overexpressing the RGS9 GAP complex [90, 91]. The light intensity threshold for transducin translocation is best explained by the membrane attachment of the $G\alpha_t$/PDE6 complex that is not free to diffuse. Thus, translocation can only occur when Gt is activated in excess of PDE6 concentration and the rate of transducin activation by R* exceeds the rate of its inactivation by the rod RGS9 GAP complex [91]. In fact, the light threshold for transducin translocation provides an additional strong argument in favor of the diffusion mechanism.

Translocation of $G\alpha_t$ and $G\beta_1\gamma_1$ to the inner compartments of rods opens up an opportunity for the transducin subunits to encounter new interaction partners. One such partner for $G\alpha_t$ is UNC119. UNC119, a mammalian ortholog of *C. elegans unc-119* [92] also known as Retina Gene 4 protein (RG4), is relatively abundant in the IS and photoreceptor synapses [93]. UNC119 was originally reported to interact with the N-terminus of the GTP-bound $G\alpha_t$ in an acylation-dependent manner [94]. Subsequently, it was shown that UNC119 can interact with heterotrimeric G_t, promote dissociation of $G\alpha_t$ from $G\beta_1\gamma_1$, and release them from the membrane [95]. The co-crystal structure of UNC119 with the lauroylated N-terminal $G\alpha_t$ peptide shows that UNC119 sequesters the lipid into the hydrophobic cavity formed by the β-sandwich fold of the protein [94]. Furthermore, UNC119 interacts with about six to ten N-terminal residues of $G\alpha_t$ [94]. Thus, UNC119 appears to disrupt the interaction of transducin subunits by sterically occluding the $G\beta_1\gamma_1$-binding site within the N-terminal α-helix of $G\alpha_t$ [96]. A recent study has estimated the relative abundance of UNC119 in rods as one molecule of UNC119 per four molecules of $G\alpha_t$ [97]. Therefore, depending on the light exposure and the extent of transducin translocation, the relative concentrations of UNC119 and $G\alpha_t$ in the inner rod compartments may range from nearly stoichiometric to a three- to fourfold excess of $G\alpha_t$. Thus, $G\alpha_t$ is likely to be a major partner of UNC119 in light-adapted rods.

The functional significance of the UNC119/Gα_t interaction is not fully known. The transport of G_t from the IS to the OS in the dark is impaired in the absence of UNC119 suggesting the protein role in the transport of transducin [94]. However, the interaction of UNC119 with Gα_t is likely to play additional regulatory or signaling roles in rods. Myristoylated proteins other than Gα_t are potential partners for UNC119. UNC119 has been shown to interact with the renal celiopathy nephronophthisis (NPHP) protein nephrocystin-3 (NPHP3) and ciliary protein cystin in a myristoyl-dependent manner [98]. Moreover, UNC119 has several known lipid-independent partners. In the IS and the connecting cilium, UNC119 binds monomeric Arf-like GTPases ARL2 and ARL3 that are involved in microtubule-dependent processes and protein trafficking through the connecting cilium [99–102]. UNC119 preferentially interacts with the GTP-bound ARL3 [100], which facilitates release of myristoylated cargo [98, 103]. Thus, UNC119 appears to serve as an ARL3 effector in ciliary transport of myristoylated proteins. In the synaptic terminal, UNC119 binds CaBP4 [104], a Ca^{2+}-binding modulator of the voltage-gated Ca^{2+} channel (Ca_v 1.4) [105], and RIBEYE, a key component of the synaptic ribbons [106]. As a major UNC119-interacting partner [97], Gα_t can outcompete myristoylated cargo from UNC119 or form ternary complexes with UNC119 and its lipid-independent partners. Since UNC119 partners have been linked to regulation of ciliary function and synaptic transmission in photoreceptor cells, translocated transducin may potentially modulate ciliary trafficking and synaptic transmission in photoreceptors. Particularly interesting in this regard is a recent study showing that transducin translocation enhances synaptic transmission from rods to rod bipolar cells [18]. In mutant mice with impaired translocation of transducin, following bright light exposure rods were less desensitized than control rods [18]. However, in these mice, rod bipolar cells were more strongly desensitized than control [18]. This sensitivity reversal suggested that transducin translocation in rods enhances signaling to rod bipolar cells. The enhancement was not attributed to modulation of IS conductances or the voltage sensitivity of the synaptic Ca^{2+} current, thereby favoring interactions of transducin with the synaptic machinery [18]. The mechanism of the rod/rod bipolar cell synapse sensitization by transducin is unknown. Hypothetically, it can be linked to Gα_t interactions with the UNC119/RIBEYE complex [106]. Alternatively, G$\beta_1\gamma_1$ can modulate neurotransmitter release through direct interactions with the SNARE complex as it has been shown for synaptic transmission in the CNS [107, 108]. Interactions of transducin outside the classical phototransduction cascade are emerging as an exciting area for future investigations.

References

1. Chabre M, Deterre P (1989) Molecular mechanism of visual transduction. Eur J Biochem 179(2):255–266
2. Pugh EN Jr, Lamb TD (1993) Amplification and kinetics of the activation steps in phototransduction. Biochim Biophys Acta 1141(2–3):111–149
3. Arshavsky VY, Lamb TD, Pugh EN Jr (2002) G proteins and phototransduction. Annu Rev Physiol 64:153–187

4. Arshavsky VY, Burns ME (2012) Photoreceptor signaling: supporting vision across a wide range of light intensities. J Biol Chem 287(3):1620–1626
5. He W, Cowan CW, Wensel TG (1998) RGS9, a GTPase accelerator for phototransduction. Neuron 20(1):95–102
6. Makino ER, Handy JW, Li T, Arshavsky VY (1999) The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein beta subunit. Proc Natl Acad Sci U S A 96(5):1947–1952
7. He W, Lu L, Zhang X, El-Hodiri HM, Chen CK, Slep KC, Simon MI, Jamrich M, Wensel TG (2000) Modules in the photoreceptor RGS9-1.Gbeta 5 L GTPase-accelerating protein complex control effector coupling, GTPase acceleration, protein folding, and stability. J Biol Chem 275(47):37093–37100
8. Cowan CW, He W, Wensel TG (2001) RGS proteins: lessons from the RGS9 subfamily. Prog Nucleic Acid Res Mol Biol 65:341–359
9. Martemyanov KA, Arshavsky VY (2009) Biology and functions of the RGS9 isoforms. Prog Mol Biol Transl Sci 86:205–227
10. Arshavsky VY, Bownds MD (1992) Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. Nature 357(6377):416–417
11. Angleson JK, Wensel TG (1994) Enhancement of rod outer segment GTPase accelerating protein activity by the inhibitory subunit of cGMP phosphodiesterase. J Biol Chem 269(23):16290–16296
12. Skiba NP, Hopp JA, Arshavsky VY (2000) The effector enzyme regulates the duration of G protein signaling in vertebrate photoreceptors by increasing the affinity between transducin and RGS protein. J Biol Chem 275(42):32716–32720
13. Brann MR, Cohen LV (1987) Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. Science 235(4788):585–587
14. Philp NJ, Chang W, Long K (1987) Light-stimulated protein movement in rod photoreceptor cells of the rat retina. FEBS Lett 225(1-2):127–132
15. Whelan JP, McGinnis JF (1988) Light-dependent subcellular movement of photoreceptor proteins. J Neurosci Res 20(2):263–270
16. Sokolov M, Lyubarsky AL, Strissel KJ, Savchenko AB, Govardovskii VI, Pugh EN Jr, Arshavsky VY (2002) Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. Neuron 34(1):95–106
17. Fain GL (2006) Why photoreceptors die (and why they don’t). Bioessays 28(4):344–354
18. Majumder A, Pahlberg J, Boyd KK, Kerov V, Kolandaivelu S, Ramamurthy V, Sampath AP, Artemyev NO (2013) Transducin translocation contributes to rod survival and enhances synaptic transmission from rods to rod bipolar cells. Proc Natl Acad Sci U S A 110:1268–1273
19. Francis SH, Turko IV, Corbin JD (2001) Cyclic nucleotide phosphodiesterases: relating structure and function. Prog Nucleic Acid Res Mol Biol 65:1–52
20. Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev 58(3):488–520
21. Baehr W, Devlin MJ, Applebury ML (1979) Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments. J Biol Chem 254(22):11669–11677
22. Hurley JB, Stryer L (1982) Purification and characterization of the gamma regulatory subunit of the cyclic GMP phosphodiesterase from retinal rod outer segments. J Biol Chem 257(18):11094–11099
23. Ovchinnikov Yu A, Lipkin VM, Kumarev VP, Gubanov VV, Khramtsov NV, Akhmedov NB, Zagranichny VE, Muradov KG (1986) Cyclic GMP phosphodiesterase from cattle retina. Amino acid sequence of the gamma-subunit and nucleotide sequence of the corresponding cDNA. FEBS Lett 204(2):288–292
24. Ovchinnikov Yu A, Gubanov VV, Khramtsov NV, Ischenko KA, Zagranichny VE, Muradov KG, Shuvaeva TM, Lipkin VM (1987) Cyclic GMP phosphodiesterase from bovine retina. Amino acid sequence of the alpha-subunit and nucleotide sequence of the corresponding cDNA. FEBS Lett 223(1):169–173

25. Deterre P, Bigay J, Forquet F, Robert M, Chabre M (1988) cGMP phosphodiesterase of retinal rods is regulated by two inhibitory subunits. Proc Natl Acad Sci U S A 85(8):2424–2428
26. Fung BK, Young JH, Yamane HK, Griswold-Prenner I (1990) Subunit stoichiometry of retinal rod cGMP phosphodiesterase. Biochemistry 29(11):2657–2664
27. Lipkin VM, Khramtsov NV, Vasilevskaya IA, Atabekova NV, Muradov KG, Gubanov VV, Li T, Johnston JP, Volpp KJ, Applebury ML (1990) Beta-subunit of bovine rod photoreceptor cGMP phosphodiesterase. Comparison with the phosphodiesterase family. J Biol Chem 265(22):12955–12959
28. Li TS, Volpp K, Applebury ML (1990) Bovine cone photoreceptor cGMP phosphodiesterase structure deduced from a cDNA clone. Proc Natl Acad Sci U S A 87(1):293–297
29. Hamilton SE, Hurley JB (1990) A phosphodiesterase inhibitor specific to a subset of bovine retinal cones. J Biol Chem 265(19):11259–11264
30. Cote RH (2004) Characteristics of photoreceptor PDE (PDE6): similarities and differences to PDE5. Int J Impot Res 16(Suppl 1):S28–S33
31. Muradov H, Boyd KK, Artemyev NO (2004) Structural determinants of the PDE6 GAF A domain for binding the inhibitory gamma-subunit and noncatalytic cGMP. Vision Res 44(21):2437–2444
32. Martinez SE, Heikaus CC, Klevit RE, Beavo JA (2008) The structure of the GAF A domain from phosphodiesterase 6C reveals determinants of cGMP binding, a conserved binding surface, and a large cGMP-dependent conformational change. J Biol Chem 283(38):25913–25919
33. Sung BJ, Hwang KY, Jeon YH, Lee JI, Heo YS, Kim JH, Moon J, Yoon JM, Hyun YL, Kim E, Eum SJ, Park SY, Lee JO, Lee TG, Ro S, Cho JM (2003) Structure of the catalytic domain of human phosphodiesterase 5 with bound drug molecules. Nature 425(6953):98–102
34. Huai Q, Liu Y, Francis SH, Corbin JD, Ke H (2004) Crystal structures of phosphodiesterases 4 and 5 in complex with inhibitor 3-isobutyl-1-methylxanthine suggest a conformation determinant of inhibitor selectivity. J Biol Chem 279(13):13095–13101
35. Barren B, Gakhar L, Muradov H, Boyd KK, Ramaswamy S, Artemyev NO (2009) Structural basis of phosphodiesterase 6 inhibition by the C-terminal region of the gamma-subunit. EMBO J 28(22):3613–3622
36. Artemyev NO, Hamm HE (1992) Two-site high-affinity interaction between inhibitory and catalytic subunits of rod cyclic GMP phosphodiesterase. Biochem J 283(Pt 1):273–279
37. Skiba NP, Artemyev NO, Hamm HE (1995) The carboxyl terminus of the gamma-subunit of rod cGMP phosphodiesterase contains distinct sites of interaction with the enzyme catalytic subunits and the alpha-subunit of transducin. J Biol Chem 270(22):13210–13215
38. Takemoto DJ, Hurt D, Oppert B, Cunnick J (1992) Domain mapping of the retinal cyclic GMP phosphodiesterase gamma-subunit. Function of the domains encoded by the three exons of the gamma-subunit gene. Biochem J 281(Pt 3):637–643
39. Artemyev NO, Natochin M, Busman M, Schey KL, Hamm HE (1996) Mechanism of photoreceptor cGMP phosphodiesterase inhibition by its gamma-subunits. Proc Natl Acad Sci U S A 93(11):5407–5412
40. Granovsky AE, Natochin M, Artemyev NO (1997) The gamma subunit of rod cGMP-phosphodiesterase blocks the enzyme catalytic site. J Biol Chem 272(18):11686–11689
41. Mou H, Cote RH (2001) The catalytic and GAF domains of the rod cGMP phosphodiesterase (PDE6) heterodimer are regulated by distinct regions of its inhibitory gamma subunit. J Biol Chem 276(29):27527–27534
42. Muradov KG, Granovsky AE, Schey KL, Artemyev NO (2002) Direct interaction of the inhibitory gamma-subunit of Rod cGMP phosphodiesterase (PDE6) with the PDE6 GAFa domains. Biochemistry 41(12):3884–3390
43. Guo LW, Muradov H, Hajipour AR, Sievert MK, Artemyev NO, Ruoho AE (2006) The inhibitory gamma subunit of the rod cGMP phosphodiesterase binds the catalytic subunits in an extended linear structure. J Biol Chem 281(22):15412–15422

44. Song J, Guo LW, Muradov H, Artemyev NO, Ruoho AE, Markley JL (2008) Intrinsically disordered gamma-subunit of cGMP phosphodiesterase encodes functionally relevant transient secondary and tertiary structure. Proc Natl Acad Sci U S A 105(5):1505–1510
45. Kameni Tcheudji JF, Lebeau L, Virmaux N, Maftei CG, Cote RH, Lugnier C, Schultz P (2001) Molecular organization of bovine rod cGMP-phosphodiesterase 6. J Mol Biol 310(4):781–791
46. Zhang X, Cote RH (2005) cGMP signaling in vertebrate retinal photoreceptor cells. Front Biosci 10:1191–1204
47. Granovsky AE, Artemyev NO (2000) Identification of the gamma subunit-interacting residues on photoreceptor cGMP phosphodiesterase, PDE6alpha '. J Biol Chem 275(52):41258–41262
48. Granovsky AE, Artemyev NO (2001) Partial reconstitution of photoreceptor cGMP phosphodiesterase characteristics in cGMP phosphodiesterase-5. J Biol Chem 276(24):21698–21703
49. Granovsky AE, Artemyev NO (2001) A conformational switch in the inhibitory gamma-subunit of PDE6 upon enzyme activation by transducin. Biochemistry 40(44):13209–13215
50. Neubert TA, Johnson RS, Hurley JB, Walsh KA (1992) The rod transducin alpha subunit amino terminus is heterogeneously fatty acylated. J Biol Chem 267(26):18274–18277
51. Bigay J, Faurobert E, Franco M, Chabre M (1994) Roles of lipid modifications of transducin subunits in their GDP-dependent association and membrane binding. Biochemistry 33(47):14081–14090
52. Kerov V, Artemyev NO (2011) Diffusion and light-dependent compartmentalization of transducin. Mol Cell Neurosci 46(1):340–346
53. Catty P, Pfister C, Bruckert F, Deterre P (1992) The cGMP phosphodiesterase-transducin complex of retinal rods. Membrane binding and subunits interactions. J Biol Chem 267(27):19489–19493
54. Clerc A, Bennett N (1992) Activated cGMP phosphodiesterase of retinal rods. A complex with transducin alpha subunit. J Biol Chem 267(10):6620–6627
55. Anant JS, Ong OC, Xie HY, Clarke S, O'Brien PJ, Fung BK (1992) In vivo differential prenylation of retinal cyclic GMP phosphodiesterase catalytic subunits. J Biol Chem 267(2):687–690
56. Faurobert E, Otto-Bruc A, Chardin P, Chabre M (1993) Tryptophan W207 in transducin T alpha is the fluorescence sensor of the G protein activation switch and is involved in the effector binding. EMBO J 12(11):4191–4198
57. Skiba NP, Bae H, Hamm HE (1996) Mapping of effector binding sites of transducin alpha-subunit using G alpha t/G alpha i1 chimeras. J Biol Chem 271(1):413–424
58. Natochin M, Granovsky AE, Artemyev NO (1998) Identification of effector residues on photoreceptor G protein, transducin. J Biol Chem 273(34):21808–21815
59. Slep KC, Kercher MA, He W, Cowan CW, Wensel TG, Sigler PB (2001) Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 A. Nature 409(6823):1071–1077
60. Hingorani VN, Tobias DT, Henderson JT, Ho YK (1988) Chemical cross-linking of bovine retinal transducin and cGMP phosphodiesterase. J Biol Chem 263(14):6916–6926
61. Clerc A, Catty P, Bennett N (1992) Interaction between cGMP-phosphodiesterase and transducin alpha-subunit in retinal rods. A cross-linking study. J Biol Chem 267(28):19948–19953
62. Leskov IB, Klenchin VA, Handy JW, Whitlock GG, Govardovskii VI, Bownds MD, Lamb TD, Pugh EN Jr, Arshavsky VY (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. Neuron 27(3):525–537
63. Bruckert F, Catty P, Deterre P, Pfister C (1994) Activation of phosphodiesterase by transducin in bovine rod outer segments: characteristics of the successive binding of two transducins. Biochemistry 33(42):12625–12634
64. Melia TJ, Malinski JA, He F, Wensel TG (2000) Enhancement of phototransduction protein interactions by lipid surfaces. J Biol Chem 275(5):3535–3542
65. Norton AW, D'Amours MR, Grazio HJ, Hebert TL, Cote RH (2000) Mechanism of transducin activation of frog rod photoreceptor phosphodiesterase. Allosteric interactiona between

the inhibitory gamma subunit and the noncatalytic cGMP-binding sites. J Biol Chem 275(49):38611–38619
66. Berger AL, Cerione RA, Erickson JW (1999) Delineation of two functionally distinct gammaPDE binding sites on the bovine retinal cGMP phosphodiesterase by a mutant gammaPDE subunit. Biochemistry 38(4):1293–1299
67. Muradov H, Boyd KK, Artemyev NO (2010) Rod phosphodiesterase-6 PDE6A and PDE6B subunits are enzymatically equivalent. J Biol Chem 285(51):39828–39834
68. Hu G, Wensel TG (2002) R9AP, a membrane anchor for the photoreceptor GTPase accelerating protein, RGS9-1. Proc Natl Acad Sci U S A 99(15):9755–9760
69. Lishko PV, Martemyanov KA, Hopp JA, Arshavsky VY (2002) Specific binding of RGS9-Gbeta 5 L to protein anchor in photoreceptor membranes greatly enhances its catalytic activity. J Biol Chem 277(27):24376–24381
70. Hu G, Zhang Z, Wensel TG (2003) Activation of RGS9-1GTPase acceleration by its membrane anchor, R9AP. J Biol Chem 278(16):14550–14554
71. Wensel TG (2008) Signal transducing membrane complexes of photoreceptor outer segments. Vision Res 48(20):2052–2061
72. Noel JP, Hamm HE, Sigler PB (1993) The 2.2 A crystal structure of transducin-alpha complexed with GTP gamma S. Nature 366(6456):654–663
73. Lambright DG, Noel JP, Hamm HE, Sigler PB (1994) Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. Nature 369(6482):621–628
74. Otto-Bruc A, Antonny B, Vuong TM, Chardin P, Chabre M (1993) Interaction between the retinal cyclic GMP phosphodiesterase inhibitor and transducin. Kinetics and affinity studies. Biochemistry 32(33):8636–8645
75. Artemyev NO, Mills JS, Thornburg KR, Knapp DR, Schey KL, Hamm HE (1993) A site on transducin alpha-subunit of interaction with the polycationic region of cGMP phosphodiesterase inhibitory subunit. J Biol Chem 268(31):23611–23615
76. Artemyev NO, Rarick HM, Mills JS, Skiba NP, Hamm HE (1992) Sites of interaction between rod G-protein alpha-subunit and cGMP-phosphodiesterase gamma-subunit. Implications for the phosphodiesterase activation mechanism. J Biol Chem 267(35):25067–25072
77. Guo LW, Hajipour AR, Ruoho AE (2010) Complementary interactions of the rod PDE6 inhibitory subunit with the catalytic subunits and transducin. J Biol Chem 285(20):15209–15219
78. Guo LW, Ruoho AE (2008) The retinal cGMP phosphodiesterase gamma-subunit - a chameleon. Curr Protein Pept Sci 9(6):611–625
79. Farber DB, Lolley RN (1947) Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. Science 186(4162):449–451
80. Rieke F, Baylor DA (1996) Molecular origin of continuous dark noise in rod photoreceptors. Biophys J 71(5):2553–2572
81. Tsang SH, Burns ME, Calvert PD, Gouras P, Baylor DA, Goff SP, Arshavsky VY (1998) Role for the target enzyme in deactivation of photoreceptor G protein in vivo. Science 282(5386):117–121
82. Zhang XJ, Gao XZ, Yao W, Cote RH (2012) Functional mapping of interacting regions of the photoreceptor phosphodiesterase (PDE6) gamma-subunit with PDE6 catalytic dimer, transducin, and regulator of G-protein signaling9-1 (RGS9-1). J Biol Chem 287(31):26312–26320
83. Calvert PD, Strissel KJ, Schiesser WE, Pugh EN Jr, Arshavsky VY (2006) Light-driven translocation of signaling proteins in vertebrate photoreceptors. Trends Cell Biol 16(11):560–568
84. Artemyev NO (2008) Light-dependent compartmentalization of transducin in rod photoreceptors. Mol Neurobiol 37(1):44–51
85. Slepak VZ, Hurley JB (2008) Mechanism of light-induced translocation of arrestin and transducin in photoreceptors: interaction-restricted diffusion. IUBMB Life 60(1):2–9
86. Mendez A, Lem J, Simon M, Chen J (2003) Light-dependent translocation of arrestin in the absence of rhodopsin phosphorylation and transducin signaling. J Neurosci 23(8):3124–3129
87. Nair KS, Hanson SM, Mendez A, Gurevich EV, Kennedy MJ, Shestopalov VI, Vishnivetskiy SA, Chen J, Hurley JB, Gurevich VV, Slepak VZ (2005) Light-dependent redistribution of arrestin in vertebrate rods is an energy-independent process governed by protein-protein interactions. Neuron 46(4):555–567

88. Rosenzweig DH, Nair KS, Wei J, Wang Q, Garwin G, Saari JC, Chen CK, Smrcka AV, Swaroop A, Lem J, Hurley JB, Slepak VZ (2007) Subunit dissociation and diffusion determine the subcellular localization of rod and cone transducins. J Neurosci 27(20):5484–5494
89. Herrmann R, Lobanova ES, Hammond T, Kessler C, Burns ME, Frishman LJ, Arshavsky VY (2010) Phosducin regulates transmission at the photoreceptor-to-ON-bipolar cell synapse. J Neurosci 30(9):3239–3253
90. Kerov V, Chen D, Moussaif M, Chen YJ, Chen CK, Artemyev NO (2005) Transducin activation state controls its light-dependent translocation in rod photoreceptors. J Biol Chem 280(49):41069–41076
91. Lobanova ES, Finkelstein S, Song H, Tsang SH, Chen CK, Sokolov M, Skiba NP, Arshavsky VY (2007) Transducin translocation in rods is triggered by saturation of the GTPase-activating complex. J Neurosci 27(5):1151–1160
92. Maduro M, Pilgrim D (1995) Identification and cloning of unc-119, a gene expressed in the Caenorhabditis elegans nervous system. Genetics 141(3):977–988
93. Higashide T, Murakami A, McLaren MJ, Inana G (1996) Cloning of the cDNA for a novel photoreceptor protein. J Biol Chem 271(3):1797–1804
94. Zhang H, Constantine R, Vorobiev S, Chen Y, Seetharaman J, Huang YJ, Xiao R, Montelione GT, Gerstner CD, Davis MW, Inana G, Whitby FG, Jorgensen EM, Hill CP, Tong L, Baehr W (2011) UNC119 is required for G protein trafficking in sensory neurons. Nat Neurosci 14(7):874–880
95. Gopalakrishna KN, Doddapuneni K, Boyd KK, Masuho I, Martemyanov KA, Artemyev NO (2011) Interaction of transducin with uncoordinated 119 protein (UNC119): implications for the model of transducin trafficking in rod photoreceptors. J Biol Chem 286(33):28954–28962
96. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB (1996) The 2.0 Å crystal structure of a heterotrimeric G protein. Nature 379(6563):311–319
97. Sinha S, Majumder A, Belcastro M, Sokolov M, Artemyev NO (2013) Expression and subcellular distribution of UNC119a, a protein partner of transducin alpha subunit in rod photoreceptors. Cell Signal 25(1):341–348
98. Wright KJ, Baye LM, Olivier-Mason A, Mukhopadhyay S, Sang L, Kwong M, Wang W, Pretorius PR, Sheffield VC, Sengupta P, Slusarski DC, Jackson PK (2011) An ARL3-UNC119-RP2 GTPase cycle targets myristoylated NPHP3 to the primary cilium. Genes Dev 25(22):2347–2360
99. Kobayashi A, Kubota S, Mori N, McLaren MJ, Inana G (2003) Photoreceptor synaptic protein HRG4 (UNC119) interacts with ARL2 via a putative conserved domain. FEBS Lett 534(1-3):26–32
100. Veltel S, Kravchenko A, Ismail S, Wittinghofer A (2008) Specificity of Arl2/Arl3 signaling is mediated by a ternary Arl3-effector-GAP complex. FEBS Lett 582(17):2501–2507
101. Kahn RA, Volpicelli-Daley L, Bowzard B, Shrivastava-Ranjan P, Li Y, Zhou C, Cunningham L (2005) Arf family GTPases: roles in membrane traffic and microtubule dynamics. Biochem Soc Trans 33(Pt 6):1269–1272
102. Schrick JJ, Vogel P, Abuin A, Hampton B, Rice DS (2006) ADP-ribosylation factor-like 3 is involved in kidney and photoreceptor development. Am J Pathol 168(4):1288–1298
103. Ismail SA, Chen YX, Miertzschke M, Vetter IR, Koerner C, Wittinghofer A (2012) Structural basis for Arl3-specific release of myristoylated ciliary cargo from UNC119. EMBO J 31(20):4085–4094
104. Haeseleer F (2008) Interaction and colocalization of CaBP4 and Unc119 (MRG4) in photoreceptors. Invest Ophthalmol Vis Sci 49(6):2366–2375
105. Haeseleer F, Imanishi Y, Maeda T, Possin DE, Maeda A, Lee A, Rieke F, Palczewski K (2004) Essential role of Ca2+-binding protein 4, a Cav1.4 channel regulator, in photoreceptor synaptic function. Nat Neurosci 7(10):1079–1087
106. Alpadi K, Magupalli VG, Kappel S, Koblitz L, Schwarz K, Seigel GM, Sung CH, Schmitz F (2008) RIBEYE recruits Munc119, a mammalian ortholog of the Caenorhabditis elegans protein unc119, to synaptic ribbons of photoreceptor synapses. J Biol Chem 283(39):26461–26467

107. Betke KM, Wells CA, Hamm HE (2012) GPCR mediated regulation of synaptic transmission. Prog Neurobiol 96(3):304–321
108. Yoon EJ, Gerachshenko T, Spiegelberg BD, Alford S, Hamm HE (2007) Gbetagamma interferes with Ca^{2+}-dependent binding of synaptotagmin to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. Mol Pharmacol 72(5):1210–1219
109. Cheever ML, Snyder JT, Gershburg S, Siderovski DP, Harden TK, Sondek J (2008) Crystal structure of the multifunctional Gbeta5-RGS9 complex. Nat Struct Mol Biol 15(2):155–162

Part II
Inner Retinal GPCR Signaling Pathways

Chapter 5
Interdependence Among Members of the mGluR6 G-protein Mediated Signalplex of Retinal Depolarizing Bipolar Cells

Ronald G. Gregg, Thomas A. Ray, Nazarul Hasan, Maureen A. McCall and Neal S. Peachey

Abstract Normal vision depends on signaling from photoreceptors to central visual areas via parallel pathways that are optimized for detecting increments (ON) or decrements (OFF) in light intensity. The divergence of these two pathways occurs at the first synapse. The OFF pathway is mediated via Off-bipolar cells that hyperpolarize in response to light increments because they utilize ionotropic glutamate receptors. On-bipolar cells that initiate the ON pathway utilize metabotropic glutamate receptors to signal via a G-protein cascade to the transient receptor potential melastatin 1 (TRPM1) channel, and depolarize in response to light increments. Several proteins (mGluR6, TRPM1, GPR179, RGS7, RGS11, nyctalopin, LRIT3, Gα0, Gβ3, Gβ5, and R9AP) have been shown to be required for normal functioning of the depolarizing bipolar cell cascade. Here, we use immunohistochemistry in mouse models that lack one or more of these proteins to understand their interdependency. The picture

R. G. Gregg (✉) · T. A. Ray · N. Hasan
Department of Biochemistry and Molecular Biology, University of Louisville,
A Building, Rm 616, 319 Abraham Flexner Way, 40202 Louisville, KY, USA
e-mail: ron.gregg@louisville.edu

T. A. Ray
e-mail: ray@neuro.duke.edu

N. Hasan
e-mail: n.hasan@louisville.edu

M. A. McCall · R. G. Gregg
Department of Ophthalmology and Visual Sciences, University of Louisville,
301 E. Muhammad Ali Blvd, Louisville, KY 40202, USA
e-mail: mo.mccall@louisville.edu

Anatomical Sciences and Neurobiology, University of Louisville, 40202 Louisville, KY, USA

N. S. Peachey
Cole Eye Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA
e-mail: peachen@ccf.org

Louis Stokes Cleveland VA Medical Center, Cleveland, OH 44106, USA

Department of Ophthalmology, Cleveland Clinic Lerner College of Medicine of Case
Western Reserve University, Cleveland, OH 44195, USA

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_5

that evolves is that of a large complex, in which the removal of any one element results in either delocalization of or decreased expression of other elements.

Light stimulation results in a graded change in sustained release of the neurotransmitter glutamate from photoreceptors. This chemical signal is converted into an electrical signal by two classes of postsynaptic bipolar cells (BCs) that either hyperpolarize (HBCs) or depolarize (DBCs) in response to a light increment. HBCs utilize ionotropic glutamate receptors and maintain the polarity of the photoreceptor signal. DBCs, in comparison, utilize the G-protein-coupled receptor (GPCR) metabotropic glutamate receptor type 6 (mGluR6) to modulate the transient receptor potential melastatin 1 (TRPM1) nonspecific cation channel, inverting the photoreceptor response. The members of this GPCR signal transduction cascade have been identified based on contributions from many laboratories. The focus of this review is to provide an overview of the interdependence of expression among these components of the DBC dendritic mGluR6 to TRPM1 signaling complex, or "signalplex," based on analyses of multiple mutant mouse lines and cascade components by immunohistochemistry.

Defects in most components of the mGluR6–GPCR cascade have been identified in humans and animal models using the noninvasive assay of retinal function, the electroretinogram (ERG). All of these mutants share the same ERG phenotype, a normal a-wave reflecting the function of the photoreceptors themselves, but the absence of a b-wave reflecting the loss of DBC function. An example of an ERG series for the *Nyx*^{nob}, no b-wave mouse mutant is shown in Fig. 5.1. The ERG defect indicates that nyctalopin (NYX) expression is required for normal DBC function not only in the mouse but also in human patients with complete congenital stationary night blindness (cCSNB). Other members of the DBC signalplex subsequently have been identified based on a comparable loss of the ERG b-wave. These include in mouse models the genes: *Grm6*, *Trpm1, Nyx, Gpr179, Lrit3,* $G\alpha_o$*, Gβ3, RGS7/RGS11* double knockouts, [10, 12, 14, 16, 19, 22, 23, 25, 26, 32, 34, 40, 41]; in cCSNB human patients: *NYX, GRM6, TRPM1, GPR179, LRIT3,* [1–3, 6, 11, 15, 18, 20, 21, 27, 35, 38, 39, 42, 44, 46–50] and in night-blind horse models, *Trpm1* [5].

We know that the GPCR cascade begins when a change in photoreceptor glutamate release is detected by the mGluR6 receptor and ends with the gating of the TRPM1 channel and a depolarizing response in the DBCs. How the other parts of the DBC signalplex function remains incompletely understood, in part because the detailed interaction and stoichiometry of the proteins remain to be defined. In this chapter, we describe the known interdependencies that have been inferred from immunohistochemical analyses of key components in mouse mutants where expression of one or more members of the cascade is absent. We focus in particular on the expression of proteins that form puncta at the tips of DBC dendrites, presumed to be the locus of the DBC cascade. In the case of rod DBCs, discrete puncta are visible, and we focus our analysis on these structures, which can be visualized using immunohistochemistry with confidence (Fig. 5.2). We do not include a description of

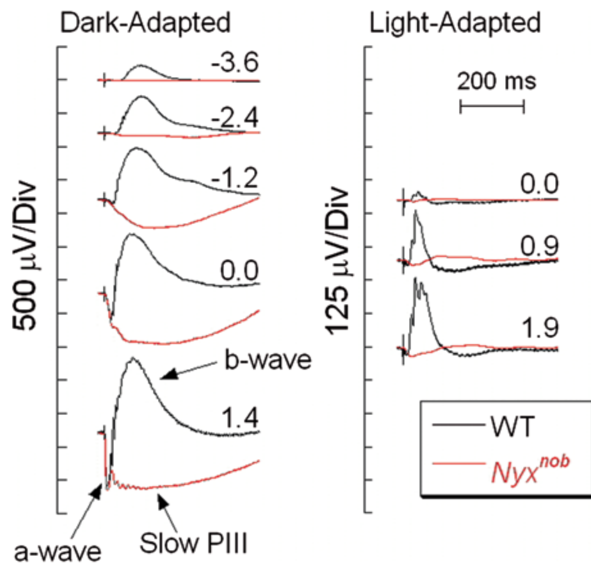

Fig. 5.1 Comparison of WT (*black traces*) and *Nyx*^{nob} (*red traces*) ERGs recorded to strobe flash stimuli presented under dark-adapted conditions (*left*) or superimposed upon a steady adapting field (*right*). WT mice generate a positive polarity b-wave that dominates the response to low luminance stimuli and follows the a-wave in response to high luminance flashes. The b-wave is absent in *Nyx*^{nob} animals. Under dark-adapted condition, the removal of the b-wave reveals slow PIII, an ERG component of Müller cell origin. Values next to each pair of waveforms indicate strobe flash luminance (log cd s/m^2)

proteins such as Gα_o or Gβ3, which do not form distinct puncta in the OPL, rather they are expressed throughout the cell [14, 43]. This does not mean they are unimportant for DBC function, as the knockout models produced for each lack an ERG b-wave and DBC function [12–14]. Rather, this feature may indicate they interact with other signalplex components in a transient manner, or are simply not visible by immunostaining when localized to DBC puncta.

Table 5.1 summarizes the expression on the tips of the DBCs of the mGluR6–GPCR components localized to the DBC puncta that have been identified in mutant mouse models to date. Below, we review results obtained in mouse mutants for *Grm6, Trpm1, Gpr179, Nyx,* or *Lrit3* in terms of the impact of a mutant allele on the expression of other signalplex components.

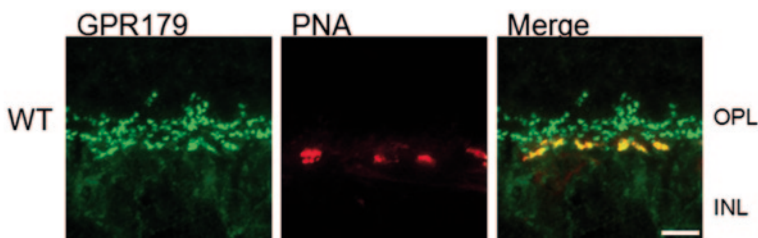

Fig. 5.2 Punctate labeling of rod DBCs in OPL of the mouse retina. Transverse section of WT mouse retina stained for GPR179 (*green*), which localizes to all DBC dendrites, and PNA (*red*) a marker for cone terminals. Note that GPR179 co-localizes with PNA. The *green puncta* in the merged image represent staining at the tips of rod DBCs

Table 5.1 Interdependence of protein expression as puncta on the dendritic tips of DBCs deduced from mutant mouse lines

Protein	Discrete puncta in OPL	*Grm 6*[(1)]	*Gna o*[(2)]	*Gnb 3*[(3)]	*Gng 13*[(4)]	*R9A P*[(5)]	*Gb 5*[(6)]	*RGS 11*[(7)]	*RGS 7*[(8)]	*RGS 7/11*[(9)]	*GPR 179*^{nob}[(10)]	*Nyx*^{nob} [(11)]	*TRPM1*^{tvrm27}[(12)]	*Trpm1*[(13)]
mGluR6	Y	−		↓		+	+	+		+	+	+	+	+
$G\alpha_o$	N		−	↓								+		
Gβ3	N			−										
Gγ13	N			↓	−									
R9AP	Y	−		↓		−		↓			−			
Gβ5	Y	−		↓		+	−	+	↓	↓	+			
RGS11	Y	↓		↓		−		−		−	−	+		
RGS7	Y	↓				+		+	−	−	−	+		
GPR179	Y	↓								+	−	+	+	
Nyctalopin	Y	+									+	−	+	+
TRPM1	Y	↓		+						+	+	−	+	−
b-wave		−	−	↓		d	−	d	d	−	−	−	−	−
Synapse (EM)		A		A			A			N/A	N	N		N

References: 1, 2—[12]; 3—[14]; 4, 5, 6, 7, 8, 9, 10—[32, 37], Fig. 5.3; 11—[33], Fig. 5.4; 12—[31]; 13—[19, 25, 33, 40], Fig. 5.3

N normal, *A* abnormal

[a] Indicates the result from the knockout mice. Protein present (+); absent (-), or (↓) on puncta at dendritic tips of DBCs.

[d] b-wave delayed. No symbol indicates data not published.

mGluR6 Expression is Required for the Localization of Multiple Signalplex Components

mGluR6 is encoded by *Grm6*, which was established as the DBC glutamate receptor using *Grm6* knockout mice, created by gene targeting [23]. These mice lack the ERG b-wave and responses to light onset in the superior colliculus. Subsequently, two additional mutants for *Grm6* were identified: *Grm6^{nob3}* [22] and *Grm6^{nob4}* [34]. Immunohistochemical results are very similar in all three lines, namely mGluR6 expression is absent from the DBC terminals. Using immunoprecipitation approaches, mGluR6 has been shown to interact with TRPM1 and GPR179 [29]. Figure 5.3 shows the consequences of the absence of mGluR6 on expression of TRPM1, GPR179, RGS11, RGS7, nyctalopin, and R9AP at the tips of DBCs. The impact on the protein level using western blots is similar (Fig. 5.4), although this does vary depending on the specific laboratory. These data and those summarized in Table 5.1 indicate that mGluR6 expression is required for the correct localization of TRPM1, RGS11, Gβ5, and R9AP to the dendritic tips of DBCs [8, 9]. The latter three components are proposed to form a trimeric GAP complex because the loss of any one results in the absence or significant reduction in expression of the other two. The western blot data indicate that the loss of mGluR6 has a relatively moderate impact on GPR179 and TRPM1. In the case of TRPM1, this is because there is a large pool of TRPM1 in the other compartments of the cell, so loss of TRPM1 from the dendrites does not appear to have a major impact on total TRPM1 expression. RGS7 puncta remain in *Grm6^{nob4}* mice [8], suggesting it is part of another complex, possibly GPR179/Gβ5/RGS7.

Localization of Other Signalplex Components is Independent of TRPM1 Expression

TRPM1 is the nonspecific cation channel modulated by the mGluR6 cascade. The first indication that TRPM1 was the channel required for DBC function came from studies in night-blind Appaloosa horses [4, 5]. In these horses, it was noted that the leopard spotting coat color and night blindness phenotypes were localized to a chromosomal region containing TRPM1. In addition, levels of *Trpm1*–mRNA expression were significantly reduced (several hundred fold) in night-blind horses, as compared to animals with normal vision. The identity of the DBC cation channel as TRPM1 was confirmed by three groups independently using knockout mice [19, 25, 40]. Their data showed that: *Trpm1$^{-/-}$* mice lack the ERG b-wave and *Trpm1$^{-/-}$* DBCs lack mGlur6-mediated light evoked responses. TRPM1 colocalizes with mGluR6 in DBC puncta, together strongly suggesting that it is a critical component of the DBC signalplex. However, unlike some other components of the complex, TRPM1 is expressed both in the puncta and throughout the entire DBC, where it is located in intracellular compartments [33]. In *Trpm1$^{-/-}$* mice, mGluR6, GPR179, RGS11, RGS7, and nyctalopin are all expressed and normally localized (Fig. 5.3).

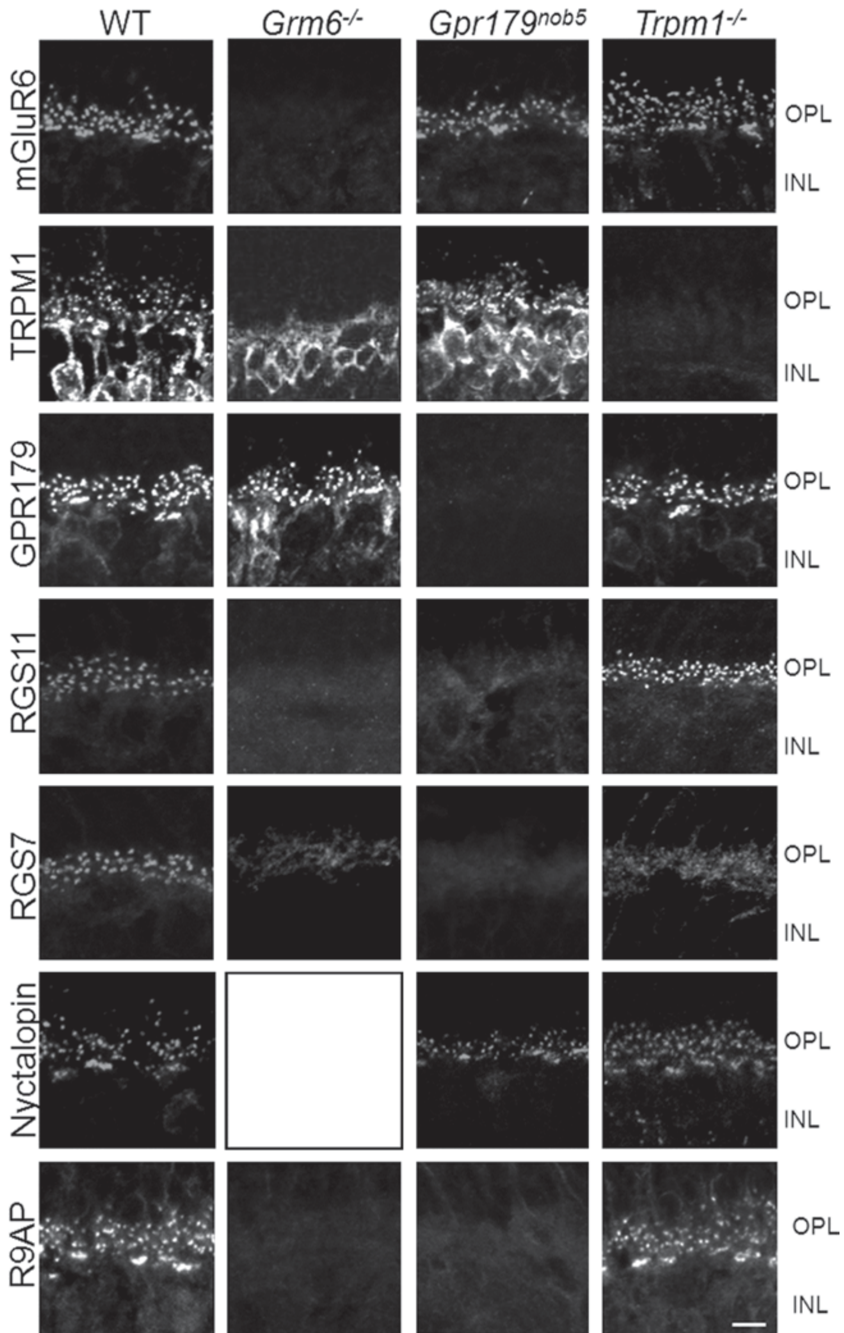

Fig. 5.3 Interdependency of DBC signalplex components revealed by immunohistochemistry in WT, *Grm6*$^{-/-}$, *GPR179*^{nob5}*,* and *Trpm1*$^{-/-}$mice. Staining in most panels was generated in the authors' laboratory using published methods and antibodies [28, 32, 33]. The image of TRPM1 staining in the *Grm6*$^{-/-}$mice is adapted from Xu et al. [45]. *Open box* indicates that this particular experiment has not been done

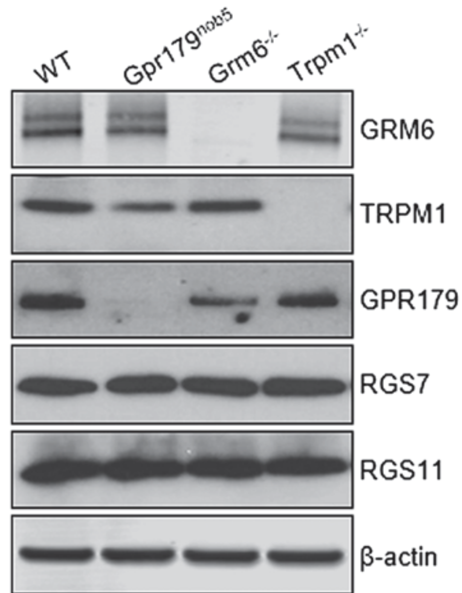

Fig. 5.4 Western blot of key DBC cascade proteins in mutant mouse lines. Western blots show the impact of eliminating three key elements of the DBC signal transduction cascade, GPR179, mGluR6, and TRPM1, on these proteins and the RGS7 and RG11 proteins critical to DBC function. In each mutant, the only protein lost is that produced by the targeted gene. Of particular note, the RGS proteins are present at near normal levels despite their abnormal localization to the DBC dendritic tips of *GPR179*^{nob5} and *Grm6*$^{-/-}$ mice (Fig. 5.2)

While the mechanism by which TRPM1 is gated remains to be firmly established (see Nawy chapter for review), the number of functional TRPM1 channels present in the DBC signalplex may be the limiting factor with respect to the maximal amplitude of the light-evoked DBC response. This conclusion was reached after using a TRPM1 mutant, *Trpm1*^{tvrm27}, resulting from an *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen. The *Trpm1*^{tvrm27} mutation is caused by a missense mutation, p.A1068T, in the predicted pore region of the channel, which is presumed to cause the lack of function. As predicted, mice homozygous for the *Trpm1*^{tvrm27} share the same ERG phenotype with other *Trpm1* knockout lines, namely the lack of a b-wave. In contrast to *Trpm1*$^{-/-}$ mice, the mutant TRPM1^{tvrm27} protein is expressed and localized correctly into puncta on the DBC dendritic tips [31].

The most significant observations from this study arose from comparisons of mice heterozygous for either the *Trpm1*^{tvrm27} or the *Trpm1* knockout allele. In *Trpm1*$^{+/-}$ mice heterozygotes, the b-wave was the same as WT controls, whereas the b-wave of *Trpm1*$^{+/tvrm27}$ heterozygous animals was about 32 % smaller. Patch-clamp recordings of *Trpm1*$^{+/tvrm27}$ heterozygous rod DBCs also showed mGluR6-mediated responses that were similarly reduced, a reduction that was not seen in heterozygous *Trpm1*$^{+/-}$ DBCs. These results suggest that the p.A1068T mutation acts as a dominant negative in the tetrameric TRPM1 channel and that the channel in the *Trpm1*$^{+/tvrm27}$ heterozygous DBCs is comprised of WT and mutant subunits. The quantitative reduction of DBC function is consistent with the hypothesis that channels with 0–2 mutant subunits retain function whereas those with 3–4 mutant subunits do not, although individual combinations of mutant and WT subunits also may have different kinetics. How the number of TRPM1 channels present in DBC

puncta might be set is unclear but likely involves yet to be discovered scaffolding components of the signalplex.

Expression of GPR179 is Required for Localization of GAP Complexes to DBC Dendrites

GPR179 is a 7-transmembrane protein. Based on primary sequence data, it has been classified as a member of the GPCR superfamily. It was discovered as an important component of the mGluR6 transduction cascade independently and simultaneously from whole-exome sequencing of patients with cCSNB [2] and from mapping the gene involved in *Gpr179*^{nob5}, a naturally occurring mouse b-wave mutant [32] and it colocalizes with DBC signalplex components (Fig. 5.3). GPR179 expression localizes both RGS7 and RGS11 to the DBC terminals (Fig. 5.3 and Orlandi et al. [28]). RGS7 is likely to interact with Gβ5, as DBCs in mice lacking Gβ5 expression also lack RGS7 expression [7, 8, 24, 36]. Because RGS7 does not interact with R9AP, it is possible that its interaction with GRP179 is critical to both its localization and perhaps function in the DBC cascade. While the specific functions of GPR179 remain to be determined, it is clear that GRP179 plays a critical role in assembling elements of the mGluR6–GPCR signalplex, although both mGluR6 and TRPM1 are localized to the tips of *Gpr179*^{nob5} DBCs (Fig. 5.3). Our recently published data indicate that GPR179 sets the sensitivity of the TRPM1 channel, whereas RGS7/RGS11 sets the sensitivity of the mGluR6 cascade [37].

Nyctalopin is Required for TRPM1 Expression

In 1998, Pardue and colleagues identified a naturally occurring no b-wave mouse mutant. In this mouse, the phenotype was inherited as an X-linked trait [30] and subsequently we showed that it was caused by a deletion mutation in *Nyx* [17], the same gene that causes the X-linked form of human cCSNB [3, 35]. Nyctalopin is a member of the small leucine-rich repeat proteoglycan family of proteins. It is anchored to the cell membrane by either a single transmembrane domain or a GPI anchor in a species-dependent manner. Nyctalopin is comprised of a series of leucine-rich repeats, which are localized to the extracellular space. Efforts by several groups to make selective antibodies to nyctalopin have been unsuccessful, likely resulting from its extensive post-translational modifications. In view of this, we made a transgenic mouse line expressing an EYFP-nyctalopin fusion gene [17], which showed restricted and punctate expression of GFP to DBC terminals and colocalization with mGluR6 puncta. When these transgenic mice were crossed onto the *Nyx*^{nob} background, the expression of the EYFP-nyctalopin fusion protein restored the ERG b-wave. In addition to the absence of the ERG b-wave, results show nyctalopin also interacts with TRPM1 [9, 33]. Together, these results suggest

that *Nyx*^{nob} DBC dysfunction is due to the loss of the TRPM1 channel from the signalplex (Fig. 5.5). How nyctalopin controls TRPM1 expression and localization to the DBC dendritic tips is unclear, but could reflect a role in trafficking TRPM1 or stabilization in the DBC membrane.

LRIT3 is Required for DBC Function

LRIT3 (leucine-rich-repeat, immunoglobulin-like and transmembrane-domain 3 (LRIT3) is the most recently identified member of the mGluR6 signalplex [47]. This discovery was made by whole-exome sequence analysis of cCSNB patients who did not harbor a mutation in any of the known members of the signalplex. *Lrit3* knockout mice lack the ERG b-wave [26] supporting the results from cCSNB patients. While the function of LRIT3 is currently unknown, it is predicted to be an extracellular protein tethered to the membrane by a single transmembrane domain. The extracellular domain contains a LRR domain similar to nyctalopin. Whether LRIT3 interacts with nyctalopin via this domain will be of interest. This similarity also suggests it may be involved in trafficking and/or localization of some critical component of the DBC signalplex, similar to nyctalopin.

Interdependent Expression of RGS11, Gβ5, and R9AP

The DBC light evoked response requires the inactivation of the mGluR6 mediated G-protein cascade. Critical to this process are regulators of G-protein signaling (RGS), protein complexes that act as GTPase-activating proteins (GAPs). Two RGS proteins, RGS7 and RGS11 appear to have redundant function, as expression of both must be eliminated to produce a no b-wave ERG phenotype [10, 41]. In photoreceptors, RGS proteins form complexes with R9AP and Gβ5. In DBCs, this is true at least for RGS11, since *R9AP*$^{-/-}$ mice lack normal localization of RGS11 and reduced expression of Gβ5. Knockout of RGS7 and RGS11 or R9AP leave the primary members of the signalplex, mGluR6, TRPM1, and GPR179 localized correctly (Fig. 5.5).

General Conclusions

This review presents the analyses of a large number of knockout mouse lines using immunohistochemical approaches to examine the interdependent/independence of expression patterns of many components of the mGluR6–GPCR transduction cascade. We believe that the results suggest that the mGluR6–GPCR signalplex is comprised of several subdomains with clear hierarchies. One domain includes

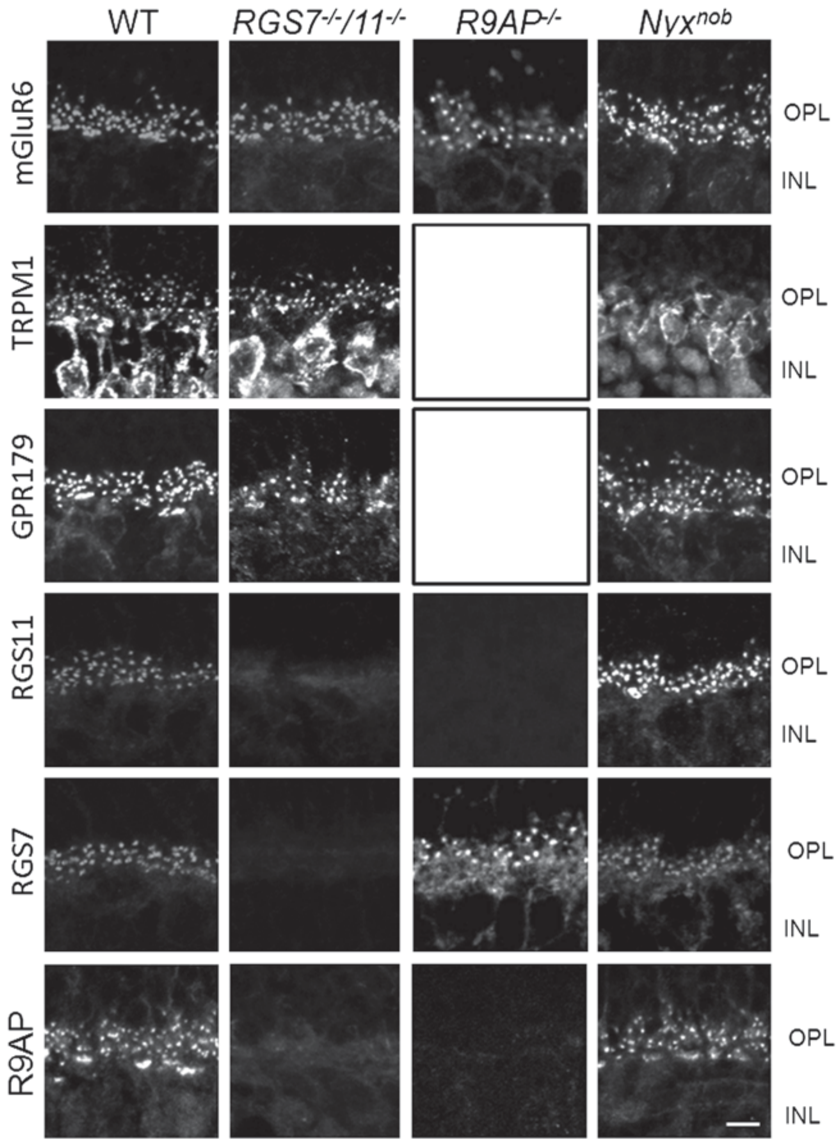

Fig. 5.5 Interdependency of DBC signalplex components revealed by immunohistochemistry in WT, *RGS7*$^{-/-}$*–/–RGS11*$^{-/-}$, *R9AP*$^{-/-}$, and *Nyx*^{nob} mice. Data for *R9AP*$^{-/-}$ mice adapted from Cao et al. [8]. Open boxes indicate that these particular experiments have not been done

mGluR6, TRPM1, R9AP, RGS11, and Gβ5, which appear to form a complex with several interdependencies, but in which expression of all members is dependent on the presence of mGluR6. A second domain includes R9AP, RGS7, and Gβ5, as the elimination of any of these has no impact on mGluR6 expression. We think that RGS7 and Gβ5 form a third complex with GPR179 and that GPR179 appears to be

a master regulator of the GAP complexes, as its absence causes mislocalization of RGS7/RGS11, R9AP, and Gβ5. Nyctalopin and TRPM1 form a separate subcomplex, and nyctalopin is essential for correct localization of the TRPM1 channel to the DBC membrane. In comparison, elimination of TRPM1 has the least impact on the expression of signalplex components, and its absence leaves the localized expression of every known component intact. This is consistent with the idea that TRPM1 gating is the final step in the DBC signal transduction process.

References

1. Audo I, Kohl S, Leroy BP, Munier FL, Guillonneau X et al (2009) TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 85:720–729
2. Audo I, Bujakowska K, Orhan E, Poloschek CM, Defoort-Dhellemmes S et al (2012) Whole-exome sequencing identifies mutations in gpr179 leading to autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 90:321–330
3. Bech-Hansen NT, Naylor MJ, Maybaum TA, Sparkes RL, Koop B et al (2000) Mutations in NYX, encoding the leucine-rich proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. Nat Genet 26:319–323
4. Bellone RR, Brooks SA, Sandmeyer L, Murphy BA, Forsyth G et al (2008) Differential gene expression of TRPM1, the potential cause of congenital stationary night blindness and coat spotting patterns (LP) in the Appaloosa horse (*Equus caballus*). Genetics 179:1861–1870
5. Bellone RR, Forsyth G, Leeb T, Archer S, Sigurdsson S et al (2010) Fine-mapping and mutation analysis of TRPM1: a candidate gene for leopard complex (LP) spotting and congenital stationary night blindness in horses. Brief Funct Genomics 9:193–207
6. Bijveld MM, Florijn RJ, Bergen AA, van den Born LI, Kamermans M et al (2013) Genotype and phenotype of 101 Dutch patients with congenital stationary night blindness. Ophthalmology 120:2072–2081
7. Cao Y, Song H, Okawa H, Sampath AP, Sokolov M, Martemyanov KA (2008) Targeting of RGS7/Gbeta5 to the dendritic tips of ON-bipolar cells is independent of its association with membrane anchor R7BP. J Neurosci 28:10443–10449
8. Cao Y, Masuho I, Okawa H, Xie K, Asami J et al (2009) Retina-specific GTPase accelerator RGS11/G beta 5S/R9AP is a constitutive heterotrimer selectively targeted to mGluR6 in ON-bipolar neurons. J Neurosci 29:9301–9313
9. Cao Y, Posokhova E, Martemyanov KA (2011) TRPM1 forms complexes with nyctalopin in vivo and accumulates in postsynaptic compartment of ON-bipolar neurons in mGluR6-dependent manner. J Neurosci 31:11521–11526
10. Cao Y, Pahlberg J, Sarria I, Kamasawa N, Sampath AP, Martemyanov KA (2012) Regulators of G protein signaling RGS7 and RGS11 determine the onset of the light response in ON bipolar neurons. Proc Natl Acad Sci U S A 109:7905–7910
11. Devi S, Markandeya Y, Maddodi N, Dhingra A, Vardi N et al (2013) Metabotropic glutamate receptor 6 signaling enhances TRPM1 calcium channel function and increases melanin content in human melanocytes. Pigment Cell Melanoma Res 26:348–356
12. Dhingra A, Lyubarsky A, Jiang M, Pugh EN Jr, Birnbaumer L et al (2000) The light response of ON bipolar neurons requires G[alpha]o. J Neurosci 20:9053–9058
13. Dhingra A, Jiang M, Wang TL, Lyubarsky A, Savchenko A et al (2002) Light response of retinal ON bipolar cells requires a specific splice variant of Galpha(o). J Neurosci 22:4878–4884
14. Dhingra A, Ramakrishnan H, Neinstein A, Fina ME, Xu Y et al (2012) Gbeta3 is required for normal light ON responses and synaptic maintenance. J Neurosci 32:11343–11355

15. Dryja TP, McGee TL, Berson EL, Fishman GA, Sandberg MA et al (2005) Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. Proc Natl Acad Sci U S A 102:4884–4889
16. Gregg RG, Mukhopadhyay S, Candille SI, Ball SL, Pardue MT et al (2003) Identification of the gene and the mutation responsible for the mouse nob phenotype. Invest Ophthalmol Vis Sci 44:378–384
17. Gregg RG, Kamermans M, Klooster J, Lukasiewicz PD, Peachey NS et al (2007) Nyctalopin expression in retinal bipolar cells restores visual function in a mouse model of complete X-linked congenital stationary night blindness. J Neurophysiol 98:3023–3033
18. Jacobi FK, Andreasson S, Langrova H, Meindl A, Zrenner E et al (2002) Phenotypic expression of the complete type of X-linked congenital stationary night blindness in patients with different mutations in the NYX gene. Graefes Arch Clin Exp Ophthalmol 240:822–828
19. Koike C, Obara T, Uriu Y, Numata T, Sanuki R et al (2010) TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. Proc Natl Acad Sci U S A 107:332–337
20. Leroy BP, Budde BS, Wittmer M, De Baere E, Berger W, Zeitz C (2009) A common NYX mutation in Flemish patients with X linked CSNB. Br J Ophthalmol 93:692–696
21. Li Z, Sergouniotis PI, Michaelides M, Mackay DS, Wright GA et al (2009) Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause complete congenital stationary night blindness in humans. Am J Hum Genet 85:711–719
22. Maddox DM, Vessey KA, Yarbrough GL, Invergo BM, Cantrell DR et al (2008) Allelic variance between GRM6 mutants, Grm6nob3 and Grm6nob4 results in differences in retinal ganglion cell visual responses. J Physiol 586:4409–4424
23. Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M et al (1995) Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. Cell 80:757–765
24. Morgans CW, Weiwei L, Wensel TG, Brown RL, Perez-Leon JA et al (2007) Gbeta5-RGS complexes co-localize with mGluR6 in retinal ON-bipolar cells. Eur J Neurosci 26:2899–2905
25. Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS et al (2009) TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proc Natl Acad Sci U S A 106:19174–19178
26. Neuille M, El Shamieh S, Orhan E, Michiels C, Antonio A et al (2014) Lrit3 Deficient Mouse (nob6): a novel model of complete congenital stationary night blindness (cCSNB). PLoS ONE 9:e90342
27. O'Connor E, Allen LE, Bradshaw K, Boylan J, Moore AT, Trump D (2006) Congenital stationary night blindness associated with mutations in GRM6 encoding glutamate receptor MGluR6. Br J Ophthalmol 90:653–654
28. Orlandi C, Posokhova E, Masuho I, Ray TA, Hasan N et al (2012) GPR158/179 regulate G protein signaling by controlling localization and activity of the RGS7 complexes. J Cell Biol 197:711–719
29. Orlandi C, Cao Y, Martemyanov KA (2013) Orphan receptor GPR179 forms macromolecular complexes with components of metabotropic signaling cascade in retina ON-bipolar neurons. Invest Ophthalmol Vis Sci 54:7153–7161
30. Pardue MT, McCall MA, LaVail MM, Gregg RG, Peachey NS (1998) A naturally occurring mouse model of X-linked congenital stationary night blindness. Invest Ophthalmol Vis Sci 39:2443–2449
31. Peachey NS, Pearring JN, Bojang P Jr, Hirschtritt ME, Sturgill-Short G et al (2012a) Depolarizing bipolar cell dysfunction due to a Trpm1 point mutation. J Neurophysiol 108:2442–2451
32. Peachey NS, Ray TA, Florijn R, Rowe LB, Sjoerdsma T et al (2012b) GPR179 is required for depolarizing bipolar cell function and is mutated in autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 90:331–339
33. Pearring JN, Bojang P, Shen Y, Koike C, Furukawa T et al (2011) A role for nyctalopin, a small leucine-rich repeat protein, in localizing the TRP melastatin 1 channel to retinal depolarizing bipolar cell dendrites. J Neurosci 31:10060–10066

34. Pinto LH, Vitaterna MH, Shimomura K, Siepka SM, Balannik V et al (2007) Generation, identification and functional characterization of the nob4 mutation of Grm6 in the mouse. Vis Neurosci 24:111–123
35. Pusch CM, Zeitz C, Brandau O, Pesch K, Achatz H et al (2000) The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. Nat Genet 26:324–327
36. Rao A, Dallman R, Henderson S, Chen CK (2007) Gbeta5 is required for normal light responses and morphology of retinal ON-bipolar cells. J Neurosci 27:14199–14204
37. Ray TA, Heath KM, Hasan N, Noel JM, Samuels IS et al (2014) GPR179 is required for high sensitivity of the mGluR6 signaling cascade in depolarizing bipolar cells. J Neurosci 30:6334–6343
38. Scholl HP, Langrova H, Pusch CM, Wissinger B, Zrenner E, Apfelstedt-Sylla E (2001) Slow and fast rod ERG pathways in patients with X-linked complete stationary night blindness carrying mutations in the NYX gene. Invest Ophthalmol Vis Sci 42:2728–2736
39. Sergouniotis PI, Robson AG, Li Z, Devery S, Holder GE et al (2012) A phenotypic study of congenital stationary night blindness (CSNB) associated with mutations in the GRM6 gene. Acta Ophthalmol 90:e192–e197
40. Shen Y, Heimel JA, Kamermans M, Peachey NS, Gregg RG, Nawy S (2009) A transient receptor potential-like channel mediates synaptic transmission in rod bipolar cells. J Neurosci 29:6088–6093
41. Shim H, Wang CT, Chen YL, Chau VQ, Fu KG et al (2012) Defective retinal depolarizing bipolar cells in regulators of G protein signaling (RGS) 7 and 11 double null mice. J Biol Chem 287:14873–14879
42. van Genderen MM,B, Pearring JN et al (2009) Mutations in TRPM1 are a common cause of complete congenital stationary night blindness. Am J Hum Genet 85:730–736
43. Vardi N, Matesic DF, Manning DR, Liebman PA, Sterling P (1993) Identification of a G-protein in depolarizing rod bipolar cells. Vis Neurosci 10:473–478
44. Xu X, Li S, Xiao X, Wang P, Guo X, Zhang Q (2009) Sequence variations of GRM6 in patients with high myopia. Mol Vis 15:2094–2100
45. Xu Y, Dhingra A, Fina ME, Koike C, Furukawa T, Vardi N (2012) mGluR6 deletion renders the TRPM1 channel in retina inactive. J Neurophysiol 107:948–957
46. Zeitz C, Minotti R, Feil S, Matyas G, Cremers FP et al (2005a) Novel mutations in CACNA1F and NYX in Dutch families with X-linked congenital stationary night blindness. Mol Vis 11:179–183
47. Zeitz C, van Genderen M, Neidhardt J, Luhmann UF, Hoeben F et al (2005b) Mutations in GRM6 cause autosomal recessive congenital stationary night blindness with a distinctive scotopic 15-Hz flicker electroretinogram. Invest Ophthalmol Vis Sci 46:4328–4335
48. Zeitz C, Jacobson SG, Hamel CP, Bujakowska K, Neuille M et al (2013) Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 92:67–75
49. Zhang Q, Xiao X, Li S, Jia X, Yang Z et al (2007) Mutations in NYX of individuals with high myopia, but without night blindness. Mol Vis 13:330–336
50. Zito I, Allen LE, Patel RJ, Meindl A, Bradshaw K et al (2003) Mutations in the CACNA1F and NYX genes in British CSNBX families. Hum Mutat 21:169

Chapter 6
Mechanistic Basis for G Protein Function in ON Bipolar Cells

Noga Vardi and Anuradha Dhingra

Abstract The synapse from photoreceptors to ON bipolar cells is unique among feedforward synapses in that it inverts the sign of the light response from hyperpolarizing in photoreceptors to depolarizing in ON bipolar cells. This occurs due to a highly specialized cascade that uses the metabotropic glutamate receptor mGluR6 to activate the heterotrimeric G protein G_o, which then closes the non-selective cation channel TRPM1. When glutamate in the synaptic cleft drops, G_o is deactivated rapidly and TRPM1 opens; this deactivation is key to the *rising phase* of the light response. This chapter presents the evidence that established $G\alpha_o$, Gβ3, and Gγ13 as G_o's subunits in ON bipolar cells. While G_o's key function is to couple mGluR6 with an effector, the different subunits also contribute to maintaining the synaptic integrity, at both the molecular and structural levels. Evidence suggests that G_o's deactivation is accelerated by at least two different types of guanosine triphosphatase (GTPase) activating protein (GAP) complexes: RGS11/Gβ5/R9AP and RGS7/Gβ5/GPR179. A third complex may contain RGS11/Gβ5/GPR179. Certain elements of these GAP complexes (RGS7 and RGS11) are redundant, but others (Gβ5 and GPR179) are crucial. Different molecules likely modulate the cascade, but so far only one, Ret-PCP2, was clearly shown to accelerate the light response in rod bipolar and certain types of ON cone bipolar cells. Interestingly, most cascade elements including mGluR6 and the GAPs are restricted to the dendritic tips, but the G protein subunits Ret-PCP2 and TRPM1 are also present in other parts of the cell.

N. Vardi (✉) · A. Dhingra
Department of Neuroscience, University of Pennsylvania, 123, Anatomy Chemistry Building, 37th and Service Drive, Philadelphia, PA 19104-6058, USA
e-mail: noga@mail.med.upenn.edu

A. Dhingra
e-mail: dhingra@mail.med.upenn.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina*, Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_6

The Heterotrimeric G Protein in the ON Bipolar Cells

Gα_o is Crucial for Coupling mGluR6 with its Downstream Cascade

In the early 1990s, several studies discovered that ON bipolar cells use a G-protein-coupled receptor to sense glutamate. This is unlike OFF bipolar cells, or most other central synapses, that use ionotropic glutamate receptors in synaptic transmission. Probing for different G protein α-subunits in ON bipolar cells using immunocytochemistry revealed staining only for Gα_o[1]. Gα_o is found in all types of ON bipolar cells (rod bipolar and ON cone bipolar cells), where it is associated with the plasma membrane and it is highly enriched in the cells' dendrites (Fig. 6.1d, f). Gα_o is not present in the ON bipolar axon terminals, or in OFF bipolar cells [2]. The evidence for Gα_o's crucial role in mediating the mGluR6 cascade rely on multiple approaches. First, in in vitro biochemical experiments using a guanosine triphosphate (GTP)-binding assay, purified mGluR6 reconstituted into lipid vesicles activates G_o much more efficiently than G_t [3]. Second, dialyzing an antibody directed against Gα_o into salamander ON bipolar cells suppresses the glutamate response in these cells, suggesting that an endogenous Gα_o-like G protein is necessary to mediate the response [4]. Third, and most convincing, electroretinography in mice lacking both splice variants of the Gα_o gene (*Gnao*) shows that this mouse has a normal negative a-wave, reflecting normal activity of photoreceptors, but no b-wave, indicating no response in ON bipolar cells. The b-wave is absent under both scotopic and photopic conditions, suggesting Gα_o is required in both rod bipolar cells and ON cone bipolar cells [5]. Under these conditions, the retina remains largely intact, and mGluR6 staining is normal. Taken together, these results establish that G_o is necessary to mediate mGluR6 signaling, and no other Gα subunit compensates for its loss in the ON bipolar cells.

ON bipolar cells express two splice variants of Gα_o, Gα_{o1} and Gα_{o2}. Using splice form-specific knockout (KO) mice and immunocytochemistry approaches, it was shown that Gα_{o1} is the predominant splice form that is present in all ON bipolar cells. Gα_{o2} is expressed at much lower levels, and it is present in rod bipolar cells and in some ON cone bipolar cells. In agreement with the localization study, mice lacking Gα_{o1} splice form display a normal electroretinogram (ERG) a-wave and lack the ERG b-wave; mice lacking the Gα_{o2} splice variant display normal a- and b-waves [6]. Although undetectable by ERG, whole-cell recordings from rod bipolar cells lacking Gα_{o1} show a small response, suggesting some contribution from Gα_{o2}. In support of this interpretation, mice lacking Gα_{o2} show reduced light sensitivity that is not due to reduction in total Gα_o, as rod bipolar cell sensitivity is not affected in heterozygous (Gα_o+/−) mice expressing 50 % of the total Gα_o level [7]. Thus, it appears that Gα_{o2} works in a coordinated manner with Gα_{o1} to improve the light sensitivity of rod bipolar cells, but the mechanism of this coordination, or how Gα_{o2} contributes, is unclear.

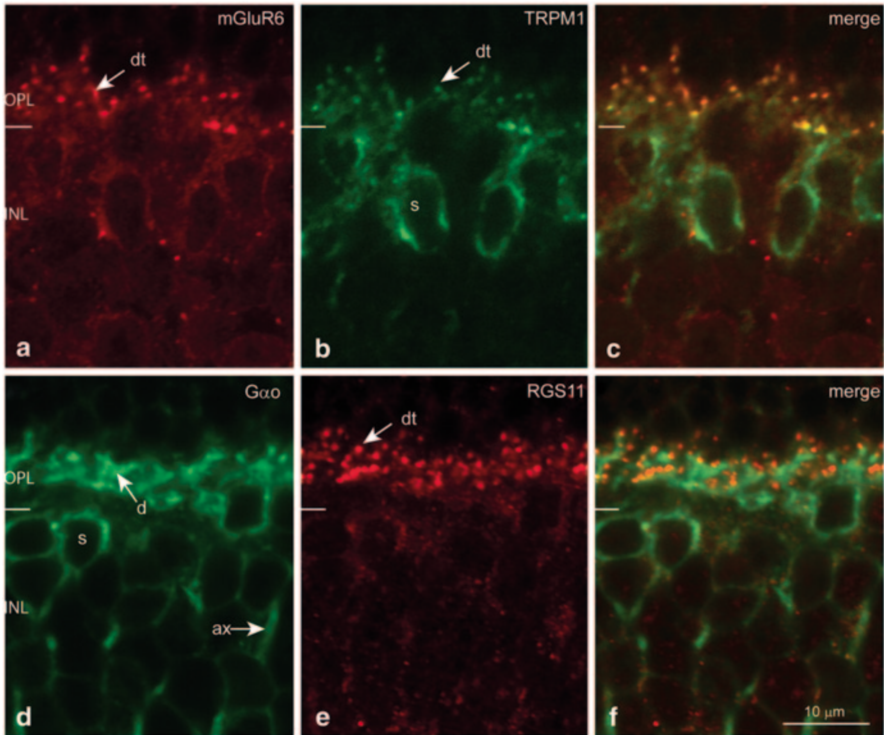

Fig. 6.1 The mGluR6 cascade components display three different staining patterns. **a–c** Double immunostaining for mGluR6 and TRPM1. Staining for mGluR6 is punctate and is restricted to the dendritic tips (*dt*) of ON bipolar cells; staining for TRPM1 is also punctate in the *OPL,* but it is also strong in the somas (*s*). **d–f** Double immunostaining for $G\alpha_o$ and RGS11. Staining for $G\alpha_o$ is strong in somas (*s*) and dendrites (*d*); the dendritic tips are not distinct. Staining for RGS11 is punctate; as for mGluR6, it is restricted to the dendritic tips (*dt*)

Gβ3 Partners with $G\alpha_o$ in Coupling mGluR6

ON bipolar cells express two Gβ subunits: Gβ3 (encoded by *Gnb3*), a conventional member of Gβ -family; and Gβ5 (encoded by *Gnb5*), a divergent member of the family. The expression of Gβ3 in ON bipolar cells has been shown over the years by different methods, in different species, and using different antibodies. First, a study investigating G proteins in retina showed Gβ3 to be in cones and in certain ON bipolar cells [8]. Second, single-cell reverse transcription polymerase chain reaction (RT-PCR) from rod bipolar cells and type 7 ON cone bipolar cells (using a transgenic mouse expressing green fluorescent protein (GFP) in these cells) showed that the message is present in these cells [9]. Third, a comparative study across vertebrate retinas showed expression in cones and in islet-positive cone ON bipolar cells across species, indicating expression of Gβ3 in many ON bipolar cells (but the exact cell types could not be identified by this method). Fourth, using a transgenic

mouse line expressing GFP only in ON bipolar cells clearly showed that all ON types express Gβ3, but OFF bipolar cells do not. A *Gnb3*-KO mouse confirmed that this expression is specific [10].

Evidence for Gβ3's role in vision came initially from a blind chicken line that was reported in a UK commercial stock in 1987. This chicken line has a naturally occurring mutation D153del in the *Gnb3* gene, which is predicted to destabilize the protein and result in autosomally recessive retinopathy and globe enlargement (RGE) phenotype [11–14]. The birds show retinal defects soon after hatching, including disorganization of the outer plexiform layer, and abnormal location of endoplasmic reticulum in the photoreceptors associated with progressive developmental disruption of photoreceptor terminals. By 3 weeks, chickens show pupillary defects and abnormal visual behavior; and by 8 weeks, the functional vision is lost [12]. Unlike in mammals, chicken Gβ3 is expressed in both rod and cone photoreceptors; thus, the precise role of Gβ3 in bipolar cells could not be established [15].

The specific role of Gβ3 in the ON bipolar cascade became clear by testing the light responses of the *Gnb3*-KO mouse. In a dark-adapted mouse, a dim scotopic stimulus elicits no positive-going b-wave. A saturated stimulus, which elicits mixed rod- and cone-generated responses in the wild-type mice, elicits a normal a-wave with no clear positive wave in the *Gnb3* KO, suggesting a defect in ON bipolar signaling [10]. However, the negative wave in the *Gnb3* KO recovers faster than that in *Grm6* (encoding mGluR6) KO (which completely lacks the b-wave). This result indicates that a weak response in ON bipolar cells pulls the ERG wave upwards. To obtain a relatively pure b-wave, one can subtract a relatively pure a-wave, such as that provided by ERG from *Grm6*-KO mouse. For both scotopic and photopic stimuli, subtracting the ERG response of the *Grm6* KO from the corresponding response of the *Gnb3* KO reveals a residual b-wave whose amplitude is about 25 % of the wild type. The dramatic reduction of the b-wave suggests that Gβ3 contributes to the light response, but the residual response suggests that it is not absolutely necessary. Consistent with the ERG recordings, whole-cell recordings from *Gnb3*-null rod bipolar cells show a tiny light response with close to normal dark resting potential and current noise [10]. This normal resting potential and current noise stand in sharp contrast to the rod bipolar cell behavior in the *Grm6* KO where the cells rest at 15 mV more hyperpolarized potential than the wild type with higher input resistance and lower noise level [16, 17]. It is however consistent with the normal holding current and current noise seen in $G\alpha_{o1}$-null rod bipolar cells [7].

The results obtained with the *Gnb3*-KO mouse were surprising because the absence of Gβ3 was less detrimental than the absence of Gβ5. When *Gnb5* is deleted, the ERG b-wave appears similar to that of *Grm6*- or *Gnao*-KO mice, suggesting that the ON light response is totally eliminated [18]. This raises the possibility that Gβ5 is the β-partner of $G\alpha_o$. The following pieces of evidence establish that this is not the case: (1) Unlike Gβ5, the distribution of Gβ3 closely resembles that of $G\alpha_o$ and of Gγ13, the best candidate for the Gγ subunit of G_o. (2) Gβ3 deletion greatly reduces Gγ13, while Gβ5 deletion does not. (3) In co-immunoprecipitation experiments, an antibody against $G\alpha_o$ pulls down Gβ3 but not Gβ5. (4) Finally, Gβ5 is a unique member of the Gβ family; it is known to form complexes with R7 regulator of G-protein-signaling (RGS) proteins in vivo but not with Gγ subunits.

Deletion of Gβ3 has a Detrimental Effect on the Correct Localization of Cascade Components

As more mouse KO models are generated, it becomes clear that deletion of any component of the mGluR6 cascade affects the localization, and sometimes the expression, of other components of the cascade. Deleting *Gnb3* is expected to reduce expression of its G protein subunit partners because the heterotrimer is considered to be a tight complex whose subunits traffic together (reviewed by Marrari et al. [19]). However, deleting *Gnb3* unexpectedly and dramatically also affects localization of other cascade components. This includes about 40 % reduction in staining for the mGluR6 receptor and the TRPM1 channel in the dendritic tips, and about 80 % reduction in staining for RGS11, Gβ5, and R9AP, three components of the GTPase activating protein (GAP) complex. Deleting *Gnb3* also mislocalizes another channel, Kir2.4, which normally localizes to the ON bipolar dendritic tips but whose function is unknown [20, 21]. The reduction of mGluR6-related cascade proteins in the ON bipolar dendritic tips may indicate either that they form a tight complex with Gβ3 or perhaps that their trafficking to the tips is coordinated to some extent by Gβ3. Although it is currently difficult to discriminate between these two possibilities, it is noteworthy that the light response in *Gnb3* KO relative to the wild type at 3 weeks of age, just at the end of the developmental process, is greater than that of the mature mouse [10]. This suggests that additional defects occur after development, and that Gβ3 in ON bipolar cells helps to maintain the tight complex. Thus, Gβ3 in the ON bipolar cascade serves not only to couple mGluR6 to G_o's effector but also to maintain the integrity of the complex.

Gβ3 Contributes to Maintenance of the Synaptic Ultrastructure

In addition to affecting the signaling components of the mGluR6 cascade in ON bipolar cell dendrites, deletion of Gβ3 affects the ultrastructural appearance of the rod-to-rod bipolar synapse. In wild-type retina, a rod terminal forms a ribbon synapse that contacts two lateral invaginating horizontal cell processes and two central invaginating rod bipolar dendrites, forming a tetrad (Fig. 6.2b); in a typical cross section though, only one bipolar is typically seen (Fig. 6.2a, b). This characteristic synapse has several membrane specializations that include the active zone right under the ribbon, which is in apposition to the horizontal cell processes, and an electron-dense rod membrane that apposes the invaginating bipolar dendrites and envelopes them. In *Gnb3* KO, the ribbons, the horizontal cell processes, and the appositions between the rod membrane and the horizontal cell membrane appear normal. However, the bipolar dendrites are rarely present in their characteristic central location (Fig. 6.2c). The apparent reduction in invaginating rod bipolar cells has also been seen in other KO mouse models lacking mGluR6 cascade components. This includes the *nob4* mouse (with a mutation in the *Grm6* coding region) [22], *Gnb5* KO [18], and one strain of RGS7/RGS11 double-KO mouse [23]. A similar

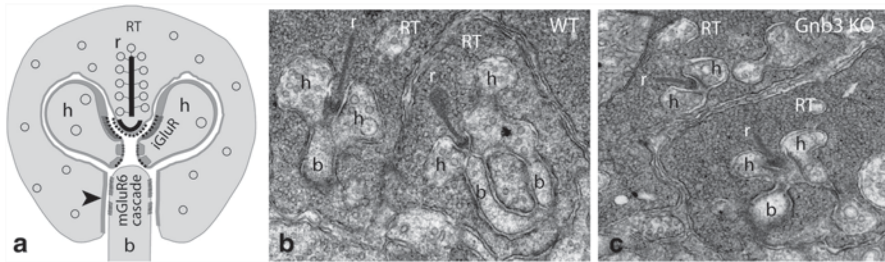

Fig. 6.2 The normal structure of the rod-to-rod bipolar cells synaptic complex and its dependency on cascade components. **a** A schematic diagram of the rod terminal (*RT*) and its postsynaptic elements. The terminal possesses an electron-dense synaptic ribbon (*r*) to which synaptic vesicles are tethered. Postsynaptic to the rod terminal are two horizontal cell processes (*h*) and two rod bipolar dendrites (*b,* typically only one is seen in an EM cross section). Horizontal cells express ionotropic receptors (*iGluR*) in apposition to the active zone, and rod bipolar cells express the metabotropic receptor mGluR6 and its cascade elements in apposition to a rod electron-dense membrane (*arrowhead*), somewhat removed from the release site. Further information about this synapse and the cone synaptic complex can be found in Vardi et al. [67]. **b** Electron micrograph image with two *RT* in the *WT* retina showing the characteristic features of the synapse. **c** Electron micrograph image with two rod terminals in the *Gnb3* null retina, the bipolar dendrites are rarely seen in the synaptic complex

deficit in the rod-to-rod bipolar cell ultrastructure is also seen in mouse KO that are missing one of the presynaptic components that are specifically localized to the apposition with the rod bipolar cells. These are pikachurin, dystroglycan, and dystrophin [24, 25]. It is likely that an absence of a critical cascade component destabilizes not only the postsynaptic elements but also some presynaptic elements via a chain of interacting molecules.

Gγ13 Contributes to the Scotopic, but Not Photopic, Light Responses

It has been suggested that Gγ13 (encoded by *Gng13*) is the third subunit that composes G_o. Like $G\alpha_o$ and Gβ3, Gγ13 is localized to all types of ON bipolar cells and not to OFF bipolar cells [9]. The message for this protein is expressed in ON bipolar cells, giving support to antibody localization [20, 26]. Supporting this idea is the fact that in vitro, Gγ13 forms a functional dimer with Gβ3 with high efficiency [27]. Furthermore, in ON bipolar cells, absence of Gβ3 or $G\alpha_{o1}$ reduces staining for Gγ13 [10, and unpublished data]. Recent results show that deleting Gγ13 reduces the ERG b-wave amplitude in response to scotopic stimuli, but not in response to photopic stimuli [28]. Furthermore, staining for GAP proteins is reduced in rod bipolar cells much more than in ON cone bipolar cells. Thus, Gγ13 greatly contributes to the generation of the scotopic light response, but it is not clear whether the main contribution is direct or indirect via regulating GAP localization, nor is it known whether another Gγ also contributes to the light response.

Diverse Functions of G Protein Subunits

An interesting outcome of the G protein KO studies in ON bipolar cells and photoreceptors is the diverse functions of the G protein subunits. Different subunits in a heterotrimer in a certain cell type may affect different proteins, and the same subunit in different cell types may contribute to different cellular processes. The following are examples of different functions of G protein subunits within the ON bipolar cells: (1) $G\alpha_{o1}$ versus $G\alpha_{o2}$: While $G\alpha_{o1}$ is the major contributor to the light response, $G\alpha_{o2}$ increases the light sensitivity of the rod bipolar cell, a function that could not be performed by a similar amount of $G\alpha_{o1}$ [7]. (2) Gβ3 versus $G\alpha_{o}$: While $G\alpha_{o}$ is absolutely required for the light response, Gβ3 greatly contributes to efficiency. Yet, Gβ3 has a greater role in maintaining correct localization of the synaptic components and its ultrastructure [5, 10, and unpublished data]. (3) Gγ13 versus $G\alpha_{o1}$ and Gβ3: Gγ13 has a smaller role both in eliciting the light response and in maintaining the synaptic integrity [unpublished data]. An example of different roles for the same subunit in different cell types is Gβ3. In cones, Gβ3 is responsible only for the correct localization of Gαt2 and Gγt2 [29], but in ON bipolar cells, Gβ3 directly or indirectly is responsible for localization of most cascade components and for normal synaptic structure. Furthermore, while Gβ3 in cones is unlikely to interact with an effector molecule, in ON bipolar cells it might modulate the TRPM1 channel gating [30]. See the chapter by Scott Nawy in the present volume.

Given this diversity, an open question is to what degree deleting Gβ3 (or Gγ13) affects the response directly by uncoupling receptor from effector, or indirectly by affecting expression and localization of other essential molecules. Unfortunately, use of conventional KO mice cannot resolve the mechanism by which the response is compromised. However, it is interesting to note that, as in ON bipolar cells, in photoreceptors, absence of, or mutations in, the Gα subunit (Gαt1 in rod or Gαt2 in cones) totally abolishes the light response, while absence of the corresponding Gβ (Gβ3 in cones) or Gγ (Gγ1 in rods) does not [29, 31–35]. Yet, absence of Gγ1 causes faster degeneration than absence of Gαt1. It is therefore natural to ask: (1) Why is deleting Gα more detrimental to the light response than deleting Gβ or Gγ, and (2) why is deleting Gβ or Gγ more detrimental to an array of cellular processes such as degeneration? The first question could be explained if photoreceptors and bipolar cells cannot compensate for the missing Gα, but they can compensate for the missing Gβ or Gγ. However, this does not explain why cellular processes are more affected after deleting Gβ or Gγ than after deleting Gα. The answer may rely on the differential roles of these subunits: Gα is absolutely required for the light response because it can be activated in the absence of Gβγ, as has been shown in vitro for rhodopsin and Gαt1 [36, 37]. Gβ and Gγ may contribute to activation efficiency, but as discussed above, they are also required for maintaining cellular processes.

The GAP Complexes in the ON Bipolar Cells

The Rising Phase of the ON Bipolar Cells' Light Response Requires Deactivation of Gα_o

To transmit a signal faithfully and repeatedly, it is necessary for any G protein to cycle between the inactive complex (G$\alpha._{GDP}$G$\beta\gamma$) and the active forms (G$\alpha._{GTP}$ + free G$\beta\gamma$). The active G$\alpha._{GTP}$ has an intrinsic GTPase activity, but this is typically slow; for Gα_o, the rate constant was estimated at around 0.3 min^{-1} [38]. In most G-protein-coupled receptor signaling cascades, the response arises with activation of the G protein and decays with GTP hydrolysis. To facilitate the decay, GTP hydrolysis is often accelerated by RGS. In ON bipolar cells, the mGluR6 cascade inverts the light response from hyperpolarizing in photoreceptors to depolarizing in these cells. In the dark, photoreceptors are depolarized, glutamate binds mGluR6, and G_o is active. This closes TRPM1 channels and maintains the cells at a hyperpolarized "resting" potential (see schematic of the mGluR6 machinery in Fig. 6.3). The light signal that hyperpolarizes the photoreceptors causes a drop of glutamate in the synaptic cleft, followed by the deactivation phase of the G protein cycle and opening of the TRPM1 channels. Therefore, in ON bipolar cells, the *rising* phase of the light response is controlled by the deactivation phase of the G protein cycle. A fast rising phase of the depolarizing light response is essential for signal transmission to third-order cells and especially to amacrine cells whose timely feedback is critical in shaping the responses in the ganglion cells. Therefore, mechanisms must exist to facilitate the rising phase of the ON bipolar cells' light responses.

An initial search for the RGS isoform in ON bipolar cells used a yeast two-hybrid screen with constitutively active Gα_o as bait [39]. This study revealed Ret-RGS1 (a splice variant of RGS20) as a candidate. Evidence supporting this candidacy includes its localization to bovine ON bipolar cells and its interaction with Gα_o in the retina. However, localization of Ret-RGS1 to ON bipolar cells in mouse could not be confirmed, and ERG light responses of RGS20 KO are similar to those of wild type (unpublished data). In contrast, two RGS proteins of the R7 family, RGS7 and RGS11, proved to contribute to Gα_o's deactivation. Interestingly, neither of these was found by the yeast two-hybrid screen. This may be explained by the subsequent findings of the obligatory GAP complex in ON bipolar cells.

Bipolar Cells Use at Least Two Different Types of GAP Complexes

Similar to phototransduction, GTP hydrolysis of the Gα protein in ON bipolar cells is accelerated by an obligatory complex that contains the R9AP anchoring protein and the unconventional Gβ5 subunit. Unlike photoreceptors (that use only RGS9), ON bipolar cells use at least two RGS proteins of the R7 family (RGS7 and RGS11), and perhaps also other molecules as discussed below. The first line of evidence for

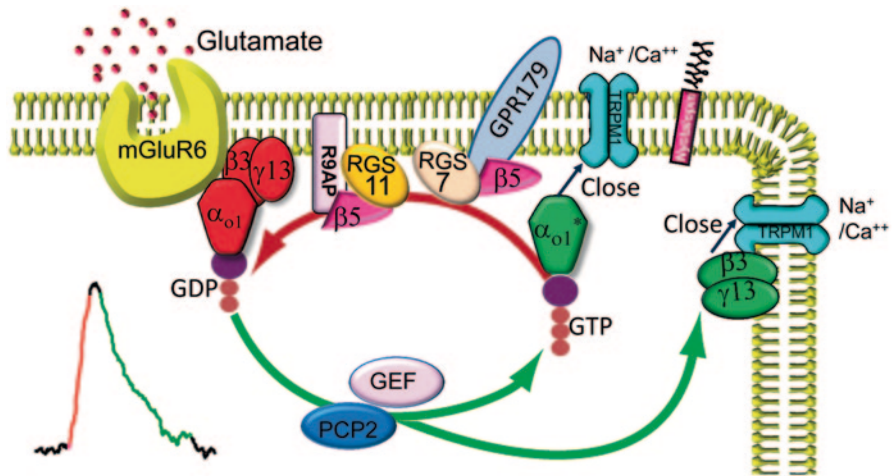

Fig. 6.3 The mGluR6 cascade and its known components. Upon binding, glutamate changes the conformation of mGluR6 and activates (*green arrows*) the G protein G_o (which is composed of $G\alpha_{o1}$, Gβ3, and Gγ13). Either $G\alpha_o$ or Gβγ closes the TRPM1 channel to keep the cell hyperpolarized. Light reduces the concentration of glutamate in the synaptic cleft, G_o deactivates (*red arrow*) by two GAP complexes, and this gives rise to the rising phase of the light response. The *inset* shows a rod bipolar light response, color coded in accordance to the activation and deactivation of G_o. *GTP* guanosine triphosphate, *GDP* guanosine diphosphate, *GEF* guanine nucleotide exchange factor

the involvement of the R7 family came from studying their localization. Immunostaining for RGS7 and RGS11 shows a punctate pattern in the OPL (see Fig. 6.1e); these stained puncta colocalize perfectly with mGluR6 staining, indicating that the proteins are restricted to the dendritic tips of rod bipolar and ON cone bipolar cells [40, 41]. A similar pattern of staining in the OPL is obtained with an antibody against R9AP and Gβ5, two components that in photoreceptors complex with RGS9 to form the obligatory GAP complex [18, 22]. Incidentally, ON bipolar cells also express an R7 binding protein, R7BP, but in these cells, R7BP does not seem to complex with RGS7 or RGS11 and may not contribute to the GTPase activity [41].

Functional evidence that RGS7 and RGS11 contribute to accelerating GTPase activity of $G\alpha_o$ is strong, but interestingly the function of these two RGSs are not additive or interdependent, but redundant. Like RGS9, RGS7 and RGS11 are thought to function as subunits in an obligatory trimer that includes Gβ5 and an anchoring protein. It appears that ON bipolar cells use two different complexes, one that contains RGS11/Gβ5/R9AP and the other containing RGS7/Gβ5/GPR179. A third complex may contain RGS11/Gβ5/GPR179. Below is a detailed account of the evidence for the involvement of the GAP complexes in the mGluR6 cascade.

Gβ5 Is Required for the Light Response and Proper Synaptic Structure

Gβ5 (*Gnb5*), a nonconventional Gβ subunit, has two splice variants: the long splice variant is specific to photoreceptors and the short variant is found in multiple areas in the brain. Gβ5 is localized to the dendritic tips of all types of ON bipolar cells, and this staining is eliminated in the *Gnb5* KO mouse [18]. Somewhat unexpectedly, the ERG b-wave in the *Gnb5* KO is completely eliminated; this suggests that Gβ5 is crucial for the light response. This result was unexpected because in photoreceptors the deletion of Gβ5 just slows down the response recovery, with the light response actually increasing in size [42]. Deletion of *Gnb5* also compromises the structure of the rod-to-rod bipolar cell synapse: While in the wild type ~35 % of synaptic profiles show at least one invaginating ON bipolar dendrite, in the KO, only ~7 % profiles show an invaginating dendrite. Thus, the lack of the b-wave in the *Gnb5* KO could be due directly to lack of GTPase activity, or to a developmental defect. However, given the known function of Gβ5 in photoreceptors, and the known interaction of Gβ5 with the R7 family [43, 44], it is more likely that the lack of response is due to absence of GAP activity. Indeed, subsequent experiments showed that co-immunoprecipitation using an antibody against RGS11 pulls down Gβ5 and R9AP from both transfected cell lines and retinal lysates [22, 40, 45]. Furthermore, deletion of Gβ5 reduces staining for, and expression of, both RGS7 and RGS11 in the dendritic tips, indicating tight complexes [22, 41].

Assuming the function of Gβ5 is not developmental, but strictly to stabilize the GAP complex and enable its activity, then why was the ON bipolar cell light response of *Gnb5*-KO mouse diminished rather than increased? The answer must lie in the unique function of the GAP in ON bipolar cells. As mentioned above, the GAP activity in ON bipolar cells mediates the rising phase of the light response, not its decay. If this necessary deactivation of $G\alpha_o$ is too slow, the response may have too little time to develop. Indeed, when both RGS7 and RGS11 are deleted, the ERG b-wave is missing, as is the rod bipolar cell flash response as measured by whole-cell recordings. However, a small response to a step of light can be detected in this dKO.

The Function of RGS7 and RGS11 Appears Redundant

Both RGS7 and RGS11 are localized to the dendritic tips of ON bipolar cells, but their concentration in rod bipolar cells compared to ON cone bipolar cells appears to be different. While staining intensity for RGS11 is similar in these two classes, staining for RGS7 is stronger in rod bipolar cells [46]. However, the functional significance of this finding is not clear since experiments so far suggest that either RGS7 or RGS11 can compensate for the loss of the other. Thus, absence of either RGS7 or RGS11 leads to a minimal delay of about 25 ms in the b-wave implicit time (time to peak of the ERG b-wave). Only when both RGS proteins are deleted is there a pronounced effect on the ON response [23, 47]. In fact, the effect on the response is so dramatic that the b-wave is completely eliminated. This likely

occurs because the short flash stimulus reduces glutamate in the synaptic cleft only for a short time, too short for GTPase activity to deactivate Gα_o and open TRPM1 channels.

Two different RGS7/RGS11 double KO models were generated; in both models, the ERG b-wave is eliminated. The resulting synaptic ultrastructure is different though. In one model, where exon 4 is deleted (resulting in truncation in the middle of the DEP domain), the percent of rod spherules with invaginating rod bipolar cells is close to normal [47], while in the other model, where exons 6–8 are deleted, the structure is deficient just as in the *Gnb5*-KO mouse [23]. The reason for this difference is not clear, but could be due to different genetic manipulations, testing at different ages, or perhaps due to the use of different cues to recognize the bipolar dendrites. It has been shown that deleting *Grm6* alters the morphology of the ON bipolar dendritic tip [48]. If a similar alteration occurs in the double KO, it can hinder structural recognition. Nonetheless, the important conclusion from both studies is that RGS7 and RGS11 are not only the main contributors to GAP activity in ON bipolar cells but also constitute essential elements in mediating the rising phase of the light response. Two more conclusions can be drawn from the RGSs studies: (1) The two RGS proteins are functionally redundant and are expressed in high abundance. This is concluded from the fact that deletion of either RGS7 or RGS11 reduces the amount of total RGS protein, yet, it hardly affects the response. (2) There may exist yet another RGS protein that contributes to the acceleration of the rising phase of the light response. This is suggested because the 40× reduction in the rate of the rising phase of the step response in the RGS7/11 dKO is smaller than what would be expected based on the intrinsic Gα_o GTPase rate [47]. An open question is why ON bipolar cells use two similar RGS proteins. A possible reason might be the uniqueness and importance of Gα_o deactivation for eliciting the light response. However, this reasoning is weak since many other components such as mGluR6 and Gα_o are crucial, and nothing compensates for their function. Alternatively, the two RGS proteins might be used together for differential fine-tuning of rod and ON cone bipolar cell responses; such fine-tuning may not be detected by ERG.

The RGS/Gβ5/GPR179: A Unique GAP Complex with Additional Functions

While both RGS7 and RGS11 belong to the R7 family of RGS proteins and both are complexed with Gβ5, only RGS11 interacts with R9AP. Furthermore, when R9AP is deleted, staining for RGS11 is almost completely lost from the dendritic tips, but that for RGS7 is without change, or may even be stronger [49]. When Gβ5 is deleted neither RGS is present in the dendritic tips and R9AP is greatly reduced [22]. Finally, when the orphan receptor GPR179 is deleted, both RGS7 and RGS11 are mislocalized [50]. Thus, there appear to be two different mechanisms for anchoring or trafficking the RGS proteins: R9AP is responsible for anchoring RGS11, and GPR179 is responsible for anchoring both RGS7 and RGS11. Beyond anchoring, R9AP and GPR179 may contribute to facilitation of the GTPase activity. R9AP was shown to enhance allosterically the GAP activity of Gα_o in oocytes expressing

mGluR6 [51]. GPR179 is also likely to contribute to enhancing GAP activity as has been shown for its close relative GPR158 [50]. However, the function of GPR179 must be very different than that of R9AP since deletion of R9AP hardly affects the ON bipolar light response, but deletion of, or mutation in, GPR179 totally eliminates the ON bipolar light response [52, 53]. The reason for total elimination of the light response after deleting GPR179 may be due to its effect on localization of both RGS7 and RGS11. If so, ON bipolar cells are predicted to give a small step response in the absence of GPR179 (as they do when both RGS7 and RGS11 are absent). Also if so, then the main role of GPR179 may be in trafficking (or stabilizing) RGS7 and RGS11. However, it is also possible that GPR179 has an RGS-independent function, a fundamental function that is independent of the GAP-related activity. Given that GPR179 has the structure of a G-protein-coupled receptor, it may be sensing ligands or ions in the synaptic cleft and relaying the signal to the bipolar cells via G_o. The precise function and its mechanism are still under investigation and results will probably provide the clue for the need of different GAP complexes.

Other Cascade Modulators

Several protein families modulate the G protein cycle. The GTPase-accelerating proteins discussed above enhance the intrinsic rate of GTP hydrolysis on Gα; guanine nucleotide exchange factors (GEFs) facilitate guanosine diphosphate (GDP)/GTP exchange; and guanine nucleotide dissociation inhibitors (GDI) inhibit the release of GDP from Gα (reviewed by Siderovski and Willard [54]). GEFs typically bind to monomeric $G\alpha_{GDP}$ competing with Gβγ. Among the known GEFs, ON bipolar cells transcribe Ric8 (also called synembryn), AGS3, and Ras-GRF1 [20, 26], but little other information is known. Theoretically, Ric8 in ON bipolar cells can work as in the *Caenorhabditis elegans'* olfactory system [55]: after activation of $G\alpha_o$ by glutamate-bound mGluR6 and after its GTP hydrolysis, Ric8 can compete with Gβγ for binding $G\alpha_{o\cdot GDP}$. It then activates $G\alpha_o$ again by re-catalyzing GTP/GDP exchange, in effect amplifying the signal to keep the cell hyperpolarized. The GDI modulators are characterized by GoLoco motifs that interact with GDP-bound $G\alpha_{i/o}$ and inhibit GDP dissociation [54, 56, 57]. Among the GDIs, the cells transcribe *Purkinje cell protein-2* (*PCP2*, also called *L7* or *GPSM4*) and *GPSM2* [20, 26]. The localization and function of PCP2 is well tested in retina and will be further discussed below. Another modulator, PKCα, is discussed in the chapter by Scott Nawy in the present volume.

PCP2 Accelerates the Light Response in ON Bipolar Cells

PCP2 has been shown in transfection systems and in the native tissue to interact with Gα of the $G_{i/o}$ family [16, 58]. This protein is considered a member of the GDI

modulators because it has the GoLoco domain, and in an in vitro system it inhibits GDP dissociation [57, 59–61]. However, a GEF function was also demonstrated [57]. PCP2 has three splice variants, the two shorter forms are expressed by Purkinje cells, and the longer variant (with an added exon at the 5′ end, termed Ret-PCP2) is expressed by rod bipolar cells and certain types of ON cone bipolar cells [62, 63]. Whole-cell recordings from rod bipolar cells showed that Ret-PCP2-null cells are more depolarized than wild-type cells with greater inward current when clamped to −60 mV. In normal Ames medium, the light response of Ret-PCP2-null rod bipolar cells is slower than that of the wild type in both the rising and the decay phases of the response. However, when inhibitory receptors are blocked, the effect of Ret-PCP2 deletion is most pronounced close to the response peak, suggesting that Ret-PCP2 sharpens the peak response. Thus, Ret-PCP2 has dual functions: It hyperpolarizes the rod bipolar cell's dark-membrane potential, and it accelerates both phases of the light response near its peak [63]. The precise mechanism of ret-PCP2's action, i.e., whether it works as a GDI or as GEF, could not be elucidated in vivo. While a GEF-like action can easily explain the results, a GDI-like action in collaboration with another GEF can also explain the results.

Contribution of Cascade Modulators to Circuit Properties

Most of the mGluR6 cascade components are expressed in all types of ON bipolar cells. Yet, different types exhibit distinct temporal bandwidths to facilitate the extraction of different temporal features in the scene. This suggests that some components may be differentially expressed in certain types of cells. Ret-PCP2 is expressed in rod bipolar cells and in several types of ON cone bipolar cells. In mouse, Ret-PCP2 is not expressed in type 7 cone bipolar cells; responses to light in this cell type are slow, perhaps because it lacks Ret-PCP2's acceleration function. Similarly, in monkey, cone bipolar cells that terminate in stratum 3 of the inner plexiform layer (DB4) express more Ret-PCP2 than those that terminate in strata 3+4 (midget bipolar cells), and these in turn express more than those that terminate in stratum 5 (DB6 and blue cone bipolar cells) [64]. This expression pattern approximates the arborization of ganglion cells (GC) with different temporal bandwidths: parasol GCs stratifying near stratum 3 display faster light responses than midget GCs stratifying in strata 3+4, and these are probably faster than sluggish-response GCs that arborize in stratum 5. This correlation once again suggests that Ret-PCP2 contributes to shaping the temporal properties of ON cone bipolar cells light responses. It is likely that more modulators will be found with differential expression in ON bipolar cells.

Differential Localization Patterns

We have described above the localization of the mGluR6 cascade components and their function. In principle, mGluR6 is needed only in the ON bipolar dendritic tips where it senses glutamate in the synaptic cleft. Similarly, the components that mediate mGluR6 action may be needed only at the tips. Indeed, most of the known components are restricted to the dendritic tips; these include mGluR6 (Fig. 6.1a), the GAPs (RGS7, RGS11, Gβ5, R9AP, and GPR179), and two other proteins known to be crucial for night vision (nyctalopin and LRIT3) whose function is not understood [65, 66]. In contrast, the G protein subunits and Ret-PCP2 are expressed throughout the cell, with strong expression in the dendrites, milder in somas, and variable in the axon terminals (Fig. 6.1d). Staining for TRPM1 shows an intermediate pattern, punctate in the OPL (suggesting little staining in the primary dendrites) and strong staining in the somas with little staining in the axons and their terminals (Fig. 6.1b). The significance of the diffused staining of the G proteins is unclear and many speculations can be raised. We suggest that a diffused staining throughout the cell may indicate a higher abundance of the protein. In the case of Ret-PCP2, the expression outside the dendrites (or abundance) was correlated with the temporal response of monkey ON cone bipolar cells [64]. It is possible, as in photoreceptors, the abundant expression of the G protein subunits is to ensure high amplification of the cascade response. For ON bipolar cells, such amplification would tend to keep the cell more hyperpolarized in the dark to maintain a large dynamic range for the depolarizing light response.

References

1. Vardi N, Matesic DF, Manning DR, Liebman PA, Sterling P (1993) Identification of a G-protein in depolarizing rod bipolar cells. Vis Neurosci 10:473–478
2. Vardi N (1998) Alpha subunit of Go localizes in the dendritic tips of ON bipolar cells. J Comp Neurol 395:43–52
3. Weng K, Lu C, Daggett LP, Kuhn R, Flor PJ, Johnson EC, Robinson PR (1997) Functional coupling of a human retinal metabotropic glutamate receptor (hmGluR6) to bovine rod transducin and rat Go in an *in vitro* reconstitution system. J Biol Chem 272:33100–33104
4. Nawy S (1999) The metabotropic receptor mGluR6 may signal through G(o), but not phosphodiesterase, in retinal bipolar cells. J Neurosci 19:2938–2944
5. Dhingra A, Lyubarsky A, Jiang M, Pugh EN Jr, Birnbaumer L, Sterling P, Vardi N (2000) The light response of ON bipolar neurons requires G[alpha]o. J Neurosci 20:9053–9058
6. Dhingra A, Jiang M, Wang TL, Lyubarsky A, Savchenko A, Bar-Yehuda T, Sterling P, Birnbaumer L, Vardi N (2002) Light response of retinal ON bipolar cells requires a specific splice variant of Galpha(o). J Neurosci 22:4878–4884
7. Okawa H, Pahlberg J, Rieke F, Birnbaumer L, Sampath AP (2010) Coordinated control of sensitivity by two splice variants of Galpha(o) in retinal ON bipolar cells. J Gen Physiol 136:443–454
8. Peng YW, Robishaw JD, Levine MA, Yau KW (1992) Retinal rods and cones have distinct G protein beta and gamma subunits. Proc Natl Acad Sci U S A 89:10882–10886
9. Huang L, Max M, Margolskee RF, Su H, Masland RH, Euler T (2003) G protein subunit G gamma 13 is coexpressed with G alpha o, G beta 3, and G beta 4 in retinal ON bipolar cells. J Comp Neurol 455:1–10

10. Dhingra A, Ramakrishnan H, Neinstein A, Fina ME, Xu Y, Li J, Chung DC, Lyubarsky A, Vardi N (2012) Gbeta3 is required for normal light on responses and synaptic maintenance. J Neurosci 32:11343–11355
11. Montiani-Ferreira F, Fischer A, Cernuda-Cernuda R, Kiupel M, DeGrip WJ, Sherry D, Cho SS, Shaw GC, Evans MG, Hocking PM, Petersen-Jones SM (2005) Detailed histopathologic characterization of the retinopathy, globe enlarged (rge) chick phenotype. Mol Vis 11:11–27
12. Tummala H, Ali M, Getty P, Hocking PM, Burt DW, Inglehearn CF, Lester DH (2006) Mutation in the guanine nucleotide-binding protein beta-3 causes retinal degeneration and embryonic mortality in chickens. Invest Ophthalmol Vis Sci 47:4714–4718
13. Montiani-Ferreira F, Shaw GC, Geller AM, Petersen-Jones SM (2007) Electroretinographic features of the retinopathy, globe enlarged (rge) chick phenotype. Mol Vis 13:553–565
14. Tummala H, Fleming S, Hocking PM, Wehner D, Naseem Z, Ali M, Inglehearn CF, Zhelev N, Lester DH (2011) The D153del mutation in GNB3 gene causes tissue specific signalling patterns and an abnormal renal morphology in Rge chickens. PLoS ONE 6:e21156
15. Ritchey ER, Bongini RE, Code KA, Zelinka C, Petersen-Jones S, Fischer AJ (2010) The pattern of expression of guanine nucleotide-binding protein beta3 in the retina is conserved across vertebrate species. Neuroscience 169:1376–1391
16. Zhang X, Zhang H, Oberdick J (2002) Conservation of the developmentally regulated dendritic localization of a Purkinje cell-specific mRNA that encodes a G-protein modulator: comparison of rodent and human Pcp2(L7) gene structure and expression. Brain Res Mol Brain Res 105:1–10
17. Xu Y, Dhingra A, Fina ME, Koike C, Furukawa T, Vardi N (2012) mGluR6 deletion renders the TRPM1 channel in retina inactive. J Neurophysiol 107:948–957
18. Rao A, Dallman R, Henderson S, Chen CK (2007) Gbeta5 is required for normal light responses and morphology of retinal ON-bipolar cells. J Neurosci 27:14199–14204
19. Marrari Y, Crouthamel M, Irannejad R, Wedegaertner PB (2007) Assembly and trafficking of heterotrimeric G proteins. Biochemistry 46:7665–7677
20. Dhingra A, Sulaiman P, Xu Y, Fina ME, Veh RW, Vardi N (2008) Probing neurochemical structure and function of retinal ON bipolar cells with a transgenic mouse. J Comp Neurol 510:484–496
21. Sulaiman P, Xu Y, Fina ME, Tummala SR, Ramakrishnan H, Dhingra A, Vardi N (2013) Kir2.4 surface expression and basal current are affected by heterotrimeric G-proteins. J Biol Chem 288:7420–7429
22. Cao Y, Masuho I, Okawa H, Xie K, Asami J, Kammermeier PJ, Maddox DM, Furukawa T, Inoue T, Sampath AP, Martemyanov KA (2009) Retina-specific GTPase accelerator RGS11/G beta 5S/R9AP is a constitutive heterotrimer selectively targeted to mGluR6 in ON-bipolar neurons. J Neurosci 29:9301–9313
23. Shim H, Wang CT, Chen YL, Chau VQ, Fu KG, Yang J, McQuiston AR, Fisher RA, Chen CK (2012) Defective retinal depolarizing bipolar cells in regulators of G protein signaling (RGS) 7 and 11 double null mice. J Biol Chem 287:14873–14879
24. Sato S, Omori Y, Katoh K, Kondo M, Kanagawa M, Miyata K, Funabiki K, Koyasu T, Kajimura N, Miyoshi T, Sawai H, Kobayashi K, Tani A, Toda T, Usukura J, Tano Y, Fujikado T, Furukawa T (2008) Pikachurin, a dystroglycan ligand, is essential for photoreceptor ribbon synapse formation. Nat Neurosci 11:923–931
25. Omori Y, Araki F, Chaya T, Kajimura N, Irie S, Terada K, Muranishi Y, Tsujii T, Ueno S, Koyasu T, Tamaki Y, Kondo M, Amano S, Furukawa T (2012) Presynaptic dystroglycan-pikachurin complex regulates the proper synaptic connection between retinal photoreceptor and bipolar cells. J Neurosci 32:6126–6137
26. Siegert S, Cabuy E, Scherf BG, Kohler H, Panda S, Le YZ, Fehling HJ, Gaidatzis D, Stadler MB, Roska B (2012) Transcriptional code and disease map for adult retinal cell types. Nat Neurosci 15:487–495
27. Poon LS, Chan AS, Wong YH (2009) Gbeta3 forms distinct dimers with specific Ggamma subunits and preferentially activates the beta3 isoform of phospholipase C. Cell Signal 21:737–744

28. Harikrishnan et al (2014) ARVO abstract 2014 (submitted)
29. Nikonov SS, Lyubarsky A, Fina ME, Nikonova ES, Sengupta A, Chinniah C, Ding XQ, Smith RG, Pugh EN Jr, Vardi N, Dhingra A (2013) Cones respond to light in the absence of transducin beta subunit. J Neurosci 33:5182–5194
30. Shen Y, Rampino MA, Carroll RC, Nawy S (2012) G-protein-mediated inhibition of the Trp channel TRPM1 requires the Gbetagamma dimer. Proc Natl Acad Sci U S A 109:8752–8757
31. Calvert PD, Krasnoperova NV, Lyubarsky A, Isayama T, Nicoló M, Kosaras B, Wong G, Gannon KS, Margolskee RF, Sidman RL, Pugh Jr EN, Makino CL, Lem J (2000) Phototransduction in transgenic mice after targeted deletion of the rod tranducin alpha-subunit. Proc Natl Acad Sci U S A 97:13913–13918
32. Chang B, Dacey MS, Hawes NL, Hitchcock PF, Milam AH, Atmaca-Sonmez P, Nusinowitz S, Heckenlively JR (2006) Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. Invest Ophthalmol Vis Sci 47:5017–5021
33. Lobanova ES, Finkelstein S, Herrmann R, Chen YM, Kessler C, Michaud NA, Trieu LH, Strissel KJ, Burns ME, Arshavsky VY (2008) Transducin gamma-subunit sets expression levels of alpha- and beta-subunits and is crucial for rod viability. J Neurosci 28:3510–3520
34. Kolesnikov AV, Rikimaru L, Hennig AK, Lukasiewicz PD, Fliesler SJ, Govardovskii VI, Kefalov VJ, Kisselev OG (2011) G-protein betagamma-complex is crucial for efficient signal amplification in vision. J Neurosci 31:8067–8077
35. Jobling AI, Vessey KA, Waugh M, Mills SA, Fletcher EL (2013) A naturally occurring mouse model of achromatopsia: characterization of the mutation in cone transducin and subsequent retinal phenotype. Invest Ophthalmol Vis Sci 54:3350–3359
36. Phillips WJ, Wong SC, Cerione RA (1992) Rhodopsin/transducin interactions. II. Influence of the transducin-beta gamma subunit complex on the coupling of the transducin-alpha subunit to rhodopsin. J Biol Chem 267:17040–17046
37. Herrmann R, Heck M, Henklein P, Hofmann KP, Ernst OP (2006) Signal transfer from GPCRs to G proteins: role of the G alpha N-terminal region in rhodopsin-transducin coupling. J Biol Chem 281:30234–30241
38. Higashijima T, Ferguson KM, Sternweis PC, Smigel MD, Gilman AG (1987) Effects of Mg2+and the beta gamma-subunit complex on the interactions of guanine nucleotides with G proteins. J Biol Chem 262:762–766
39. Dhingra A, Faurobert E, Dascal N, Sterling P, Vardi N (2004) A retinal-specific regulator of G-protein signaling interacts with Galphao and accelerates an expressed metabotropic glutamate receptor 6 cascade. J Neurosci 24:5684–5693
40. Morgans CW, Wensel TG, Brown RL, Perez-Leon JA, Bearnot B, Duvoisin RM (2007) Gbeta5-RGS complexes co-localize with mGluR6 in retinal ON-bipolar cells. Eur J Neurosci 26:2899–2905
41. Cao Y, Song H, Okawa H, Sampath AP, Sokolov M, Martemyanov KA (2008) Targeting of RGS7/Gbeta5 to the dendritic tips of ON-bipolar cells is independent of its association with membrane anchor R7BP. J Neurosci 28:10443–10449
42. Krispel CM, Chen D, Melling N, Chen YJ, Martemyanov KA, Quillinan N, Arshavsky VY, Wensel TG, Chen CK, Burns ME (2006) RGS expression rate-limits recovery of rod photoresponses. Neuron 51:409–416
43. Snow BE, Krumins AM, Brothers GM, Lee SF, Wall MA, Chung S, Mangion J, Arya S, Gilman AG, Siderovski DP (1998) A G protein gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to Gbeta5 subunits. Proc Natl Acad Sci U S A 95:13307–13312
44. Levay K, Cabrera JL, Satpaev DK, Slepak VZ (1999) Gbeta5 prevents the RGS7-Galphao interaction through binding to a distinct Ggamma-like domain found in RGS7 and other RGS proteins. Proc Natl Acad Sci U S A 96:2503–2507
45. Song JH, Song H, Wensel TG, Sokolov M, Martemyanov KA (2007) Localization and differential interaction of R7 RGS proteins with their membrane anchors R7BP and R9AP in neurons of vertebrate retina. Mol Cell Neurosci 35:311–319
46. Mojumder DK, Qian Y, Wensel TG (2009) Two R7 regulator of G-protein signaling proteins shape retinal bipolar cell signaling. J Neurosci 29:7753–7765

47. Cao Y, Pahlberg J, Sarria I, Kamasawa N, Sampath AP, Martemyanov KA (2012) Regulators of G protein signaling RGS7 and RGS11 determine the onset of the light response in ON bipolar neurons. Proc Natl Acad Sci U S A 109:7905–7910
48. Ishii M, Morigiwa K, Takao M, Nakanishi S, Fukuda Y, Mimura O, Tsukamoto Y (2009) Ectopic synaptic ribbons in dendrites of mouse retinal ON- and OFF-bipolar cells. Cell Tissue Res 338:355–375
49. Jeffrey BG, Morgans CW, Puthussery T, Wensel TG, Burke NS, Brown RL, Duvoisin RM (2010) R9AP stabilizes RGS11-G beta5 and accelerates the early light response of ON-bipolar cells. Vis Neurosci 27:9–17
50. Orlandi C, Posokhova E, Masuho I, Ray TA, Hasan N, Gregg RG, Martemyanov KA (2012) GPR158/179 regulate G protein signaling by controlling localization and activity of the RGS7 complexes. J Cell Biol 197:711–719
51. Masuho I, Celver J, Kovoor A, Martemyanov KA (2010) Membrane anchor R9AP potentiates GTPase-accelerating protein activity of RGS11 x Gbeta5 complex and accelerates inactivation of the mGluR6-G(o) signaling. J Biol Chem 285:4781–4787
52. Audo I et al (2012) Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 90:321–330
53. Peachey NS et al (2012) GPR179 is required for depolarizing bipolar cell function and is mutated in autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 90:331–339
54. Siderovski DP, Willard FS (2005) The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 1:51–66
55. Hinrichs MV, Torrejon M, Montecino M, Olate J (2012) Ric-8: different cellular roles for a heterotrimeric G-protein GEF. J Cell Biochem 113:2797–2805
56. Peterson YK, Bernard ML, Ma H, Hazard S 3rd, Graber SG, Lanier SM (2000) Stabilization of the GDP-bound conformation of Gialpha by a peptide derived from the G-protein regulatory motif of AGS3. J Biol Chem 275:33193–33196
57. Willard FS, Kimple RJ, Siderovski DP (2004) Return of the GDI: the GoLoco motif in cell division. Annu Rev Biochem 73:925–951
58. Luo Y, Denker BM (1999) Interaction of heterotrimeric G protein Galphao with Purkinje cell protein-2. Evidence for a novel nucleotide exchange factor. J Biol Chem 274:10685–10688
59. Natochin M, Gasimov KG, Artemyev NO (2001) Inhibition of GDP/GTP exchange on G alpha subunits by proteins containing G-protein regulatory motifs. Biochemistry 40:5322–5328
60. Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski DP (2002) Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. Nature 416:878–881
61. Willard FS, McCudden CR, Siderovski DP (2006) G-protein alpha subunit interaction and guanine nucleotide dissociation inhibitor activity of the dual GoLoco motif protein PCP-2 (Purkinje cell protein-2). Cell Signal 18:1226–1234
62. Berrebi AS, Oberdick J, Sangameswaran L, Christakos S, Morgan JI, Mugnaini E (1991) Cerebellar Purkinje cell markers are expressed in retinal bipolar neurons. J Comp Neurol 308:630–649
63. Xu Y, Sulaiman P, Feddersen RM, Liu J, Smith RG, Vardi N (2008) Retinal ON bipolar cells express a new PCP2 splice variant that accelerates the light response. J Neurosci 28:8873–8884
64. Sulaiman P, Fina M, Feddersen R, Vardi N (2010) Ret-PCP2 colocalizes with protein kinase C in a subset of primate ON cone bipolar cells. J Comp Neurol 518:1098–1112
65. Gregg RG, Kamermans M, Klooster J, Lukasiewicz PD, Peachey NS, Vessey KA, McCall MA (2007) Nyctalopin expression in retinal bipolar cells restores visual function in a mouse model of complete X-linked congenital stationary night blindness. J Neurophysiol 98:3023–3033
66. Zeitz C et al (2013) Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 92:67–75
67. Vardi N, Morigiwa K, Wang TL, Shi YJ, Sterling P (1998) Neurochemistry of the mammalian cone synaptic complex. Vision Res 38:1359–1369

Chapter 7
Modulation of TRPM1 and the mGluR6 Cascade in ON Bipolar Cells

Scott Nawy

Abstract In the retina, cones contact two types of bipolar cells, releasing the same transmitter onto each. Since the two types of bipolar cells must respond to light (and therefore cone transmitter) with opposite polarities, each has evolved very different postsynaptic mechanisms to solve this problem. The OFF bipolar cell expresses ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and kainate glutamate receptors, conferring depolarization during photoreceptor transmitter release. However, the requirement for a hyperpolarizing action of glutamate at ON bipolar cells ruled out the use of ionotropic receptors at this synapse. Years of investigation by a number of different laboratories have revealed this highly novel, perhaps unique mechanism by which activation of a glutamate receptor, metabotropic glutamate receptor type 6 (mGluR6), results in membrane hyperpolarization. The photoreceptor–ON bipolar cell synapse must also be adaptive, enhancing rapid changes in illumination while attenuating slower, more long-lasting changes in order to avoid saturation of downstream synapses. Calcium appears to play a major role in this form of short-term plasticity. Finally, recent evidence suggests that the gain of this synapse is additionally regulated by ambient light levels. Both cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) and protein kinase C (PKC) appear to play key roles in these forms of long-term plasticity. Sections "ON Bipolar Cells and the mGluR6 Pathway: A Brief History" and "Elucidation of the mGluR6 Signaling Cascade" of this chapter summarize what is currently known about this synaptic pathway, with an emphasis on the major historical breakthroughs that shaped our understanding of this exceedingly complex synapse along the way. Sections "Modulation of the mGluR6 Cascade: Ca^{2+}", "Modulation of the mGluR6 Cascade: cGMP", and "Modulation of the mGluR6 Cascade: PKC and DAG" summarize the roles of Ca^{2+}, cGMP, and PKC in the regulation of this pathway.

S. Nawy (✉)
Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine,
Kennedy Center Room 525, 1410 Pelham Parkway South, Bronx, NY 10461, USA
e-mail: Scott.Nawy@einstein.yu.edu

Department of Neuroscience, Albert Einstein College of Medicine,
Kennedy Center Room 525, 1410 Pelham Parkway South, Bronx, NY 10461, USA

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_7

ON Bipolar Cells and the mGluR6 Pathway: A Brief History

A Surprising Discovery: Light Depolarizes ON Bipolar Cells by Opening Channels

It has been four decades since the first published intracellular recordings of bipolar cell light responses [1–3]. This was the era of the sharp electrode, as patch clamp recording was more than a decade away. Mammalian cells were too small to penetrate with microelectrodes, and for this reason, either amphibian or fish (cyprinid) retinas were chosen as model systems for studying retinal circuitry. The earliest studies focused on the polarity of light responses, showing that cells could be classified as ON or OFF, depending upon their response to light. They also conclusively demonstrated the concept of center and surround components of the receptive field, correctly predicting that the center response of bipolar cells was provided by direct input from photoreceptors, while the surround response, which had the opposite polarity as the center response, originated from horizontal cells.

The second wave of papers began to focus on finer details of synaptic transmission. An important question to be addressed was how light could depolarize one type of bipolar cell and hyperpolarize another. It was understood that the best way to approach this question was to characterize the types of channels that were activated by light in each type of bipolar cell. The basic strategy was to change the membrane potential by passing current through the electrode during the light response. This was a daunting task when one considers that the resistance of the electrode was typically an order of magnitude higher than the input resistance of the cell. Often, a reversal of the light response was not possible, as too much current was required to change the membrane potential sufficiently, and so investigators relied on extrapolations of the *I–V* relation. By far, the most studied bipolar cell during this time period was the Mb-type ON bipolar cell from the carp or goldfish retina. It has a large cell body, making it amenable to electrophysiological studies. As will be discussed in the next section, bipolar cells in mammalian retina receive input from rods or cones, but almost never both. However, this arrangement is different in the retinas of lower vertebrates, where bipolar cells, including the Mb-type, often receive synaptic input from both rods and cones [4, 6–9]. Measurements of resistance changes and reversal potentials associated with the light response yielded a surprising result: Light responses originating from rods were associated with an increase in membrane conductance, and a reversal potential approaching 0 mV. On the other hand, light responses originating from cones, measured in the same bipolar cell, were associated with a decrease in conductance, and a reversal potential at negative voltages [10–15]. OFF bipolar cells appear to follow a simpler rule, with input from both rods and cones activating the same conductance mechanism. Activation of either rods or cones was found to decrease membrane conductance, indicating that light closed channels in OFF bipolar cells [15].

This initially seemed puzzling: When comparing input from the same photoreceptor (i.e., rods), ON and OFF bipolar cells respond with opposite polarities, so why should the reversal potential of the light response be the same for both? The answer was found to lie in the conductance change associated with the light response: OFF bipolar cells respond to light with a decrease in conductance, an indication that light closes channels, while ON bipolar cells respond with a conductance increase. Because it was known that the photoreceptor transmitter was released in darkness and suppressed by light [16], investigators suggested that the endogenous transmitter activates a Na^+ conductance in OFF bipolar cells, and decreases Na^+ conductance in ON bipolar cells. In the case of the ON bipolar cell, the effect of the photoreceptor transmitter was compared to an inhibitory transmitter, and so for this reason there was some speculation at that time, albeit only briefly, that photoreceptor might actually release different transmitters onto ON and OFF bipolar cells. Soon it would be appreciated that ON and OFF bipolar cells expressed very different kinds of glutamate receptors.

The Unique Pharmacology of the ON Bipolar Cell Synaptic Hinted at a Novel Synaptic Mechanism

The study of bipolar cells, the ON type in particular, benefited tremendously from the discovery of a selective agonist for the photoreceptor–ON bipolar cell synapse. Almost simultaneously, two papers were published demonstrating that the glutamate agonist L-2-amino-4-phosphonobutyric acid (L-AP4; known as L-APB at that time) selectively blocked light responses of ON bipolar cells [17, 18]. Measurements of conductance changes associated with L-AP4 demonstrated conclusively that it acted not as an antagonist to block the action of transmitter, but rather to mimic transmitter effects. In the mammalian retina, application of L-AP4 blocks all input along the ON pathway, regardless of whether it originates from rods or cones [19], supporting the idea that a single kind of glutamate receptor is expressed in all types of ON bipolar cells in the mammalian retina. Interestingly, in fish retina, L-AP4 acts only on the rod input to ON bipolar cells, leaving the cone input unchanged [20]. Thus, in the fish, inputs from rods and cones differ not only in their conductance mechanisms but in their pharmacology as well. In fact, cone input was later shown to be mediated by a glutamate transporter, rather than a traditional receptor-mediated pathway [21, 22]. The effectiveness of L-AP4 on all types of ON bipolar cells in the mammalian retina suggests that the transporter mechanism used to convey input from cones in lower vertebrates has been abandoned in the retina of higher-order vertebrates, and will not be considered further here.

At the end of the 1980s, the tremendous importance of G proteins in transmitter signaling was becoming clear. It seemed likely that a channel-closing transmitter would require some intracellular machinery, such as a G protein cascade, interposed between the receptor and the synaptic channel. One example which proved to be useful was the inhibition of K^+ current in bullfrog sympathetic ganglia that came

to be known as M current [23] through a mechanism that required activation of a G protein [24]. The recent advancement of patch clamp recording allowed for experiments that could directly test the possibility that closure of the synaptic channel of ON bipolar cells likewise required G protein activation. Whole-cell recordings revealed that the response to glutamate ran down rapidly, an indication that cellular constituents washed away by whole-cell recording were required for supporting the function of the channel that is closed by glutamate [25]. Soon after, it was demonstrated that the intracellular application of the hydrolysis-resistant guanosine triphosphate (GTP) analog GTP-γ-S eliminated the response to glutamate, locking the synaptic channel in a closed state [26]. Verification that the glutamate receptor expressed in ON bipolar cell dendrites was indeed a metabotropic, G-protein-coupled receptor came several years later when it was cloned and termed metabotropic glutamate receptor type 6 (mGluR6) [27].

Elucidation of the mGluR6 Signaling Cascade

Components of the mGluR Pathway

The identity of the major components of the ON bipolar cell cascade was only slowly revealed. As mentioned above, the receptor, termed mGluR6, was cloned in 1993. The G protein to which the receptor couples was identified as G_o several years later [28, 29] through a combination of genetic and physiological approaches (see Vardi and Dhingra, this volume). However, the mystery of the channel itself proved to be the most difficult of all to solve. Insight came from an unexpected place, the Appaloosa horse, where it was found that horses with abnormalities in pigmentation called leopard spot often also suffered from congenital stationary night blindness (CSNB) [30]. Leopard spot results from homozygous expression of the incomplete dominant gene LP. Bellone and colleagues found that in LP-homozygous animals, a closely associated gene, transient receptor potential melastatin 1 (*Trpm1*), was expressed in the retina at less than 1% of the level found in normal Appaloosa horses [31].

TRPM1 encodes a member of the Trp family of channels, whose physiological properties were completely unknown and undefined at that time. It was known to be expressed in melanocytes and was first identified in a cDNA screen in a search for prognostic markers for melanomas [32]. For reasons that are not clear, there is a decrease in levels of TRPM1 mRNA in primary cutaneous tumors [33]. More details about TRPM1 can be found in a recent review [34]. This discovery was followed by a series of publications showing that ON bipolar cell transduction was absent in a mouse line in which expression of TRPM1 was eliminated [35–38]. These studies strongly indicate that TRPM1 is necessary for generation of a synaptic response in ON bipolar cells, but do not eliminate the possibility that other, still unidentified channels may form a complex with TRPM1 to generate this response.

Thus, key components of the cascade including the receptor, G protein, and effector channel had been identified. The initial finding, documented some 40 years ago, that the photoreceptor transmitter hyperpolarized ON bipolar cells could now be explained, at least in a very rudimentary way. Light suppresses photoreceptor transmitter release and inactivates the mGluR6 cascade. When the cascade is shut OFF, TRPM1 can open (see below) and the net flux of Na^+ and K^+ through the channel depolarizes the cell. Conversely, in the dark, glutamate binds to mGluR6, activating the cascade, and TRPM1 closes. In the absence of the current contributed by TRPM1, the resting conductance is carried mostly by K^+, and this keeps the cell in a hyperpolarized state. This mechanism also accounts for the original observation that the light response is associated with an increase in conductance. It should be noted that expression of mGluR6, TRPM1, and G_o are common to both rods and cone bipolar cells. Thus, mammals appear to have departed from the evolutionary path of teleost, in which rod and cone input was mediated by different transduction machinery in the dendrites of ON bipolar cells.

Gating of TRPM1: What Turns It OFF, and What Turns It On?

In darkness, glutamate binds to mGluR6, causing the dissociation of the $G\alpha_o$ and $G\beta\gamma$ subunits. In principle, either the alpha subunit or the dimer could bind directly to the channel and close it. The traditional view is that $\beta\gamma$ dimers are better suited to interact with channels due to their greater hydrophobicity, and the G-protein-coupled inward rectifier potassium channels (GIRK) and Ca^{2+} channels are the best studied examples of this [39–43]. In support of this, dialysis of $G\beta\gamma$ dimers into rod bipolar cells suppresses TRPM1 and blocks the light response [38]. In that study, dialysis with $G\alpha_o$ subunits that had been preactivated with GTPγS had no obvious effect on the current. However, there is some controversy about the relative importance of $G\beta\gamma$ versus $G\alpha_o$ for closing TRPM1. Using inside-out patches of membrane excised from CHO cells that had been transfected with TRPM1, Furakawa and his colleagues demonstrated that direct application of $G\alpha_o$ to the patch closed channels thought to be TRPM1 [36]. An alternative idea that $G\alpha_o$ closes the TRPM1 channel indirectly by catalyzing the degradation of a second messenger that is required for channel opening would seem to be ruled out by experiments using isolated membranes. It is possible that tightly bound intracellular machinery remained attached to the inside-out patches, and that the $G\alpha_o$ interacted with a tightly bound cytosolic effector rather than the channel itself. It is difficult to fully reconcile these two viewpoints except to conclude that perhaps both the alpha subunit and the $\beta\gamma$ dimer may play a role in closing the channel.

During the light response, TRPM1 is open. Is a second messenger required, or is opening constitutive? To date, no second messenger has been identified, and a number of candidates have been ruled out. These include the classic second messengers cGMP, cyclic adenosine monophosphate (cAMP), Ca^{2+}, IP3, and diacylglycerol (DAG) [28, 44, 45]. Other lipid-based compounds, including endocannabinoids

such as 2-AG and anandamide, remain possible candidates. On the other hand, studies of TRPM1 in expression systems and human melanocytes consistently demonstrate constitutive activation [36, 38, 46, 47]. Although it is difficult to rule out the existence of a second messenger that is sufficiently ubiquitous as to be present in the cytosol of most cell lines and expression systems, the most parsimonious explanation would seem to be that TRPM1 can open constitutively.

The currents obtained in cell lines are substantially smaller than those found in bipolar cells. This may result from TRPM1 channels that are in a continuous state of desensitization (see below), or because expression systems lack the necessary accessory proteins for proper trafficking insertion, and maintenance in the membrane (see Gregg, Chap. 5 in the present volume). To summarize, in the dark, TRPM1 channels are closed by the action of either Gβγ dimers [38] or the dissociated $G\alpha_o$ subunit [36, 47]. Light inactivates the mGluR6 cascade by suppressing transmitter release, allowing for reassociation of the G protein complex, permitting the constitutive opening of TRPM1 and the conduction of depolarizing Na^+, K^+, and Ca^{2+} through the channel.

Modulation of the mGluR6 Cascade: Ca^{2+}

Calcium Influx Triggers "Desensitization" of TRPM1

As described above, when expressed in cell lines, TRPM1 appears to be open constitutively [34, 36, 38, 47], but in ON bipolar cells, the open state of the channel is tightly regulated. Steps of light depolarize ON bipolar cells, but in most cases this depolarization is transient compared with the stimulus, and is followed by a decay of the depolarization toward baseline. This is particularly true for higher light intensities. The kinetics of the light response are evident in the earliest recordings of bipolar cells [1, 2], and have often been attributed to the transient nature of the photoreceptor response. Rods, in particular, respond to steps of light with a pronounced peak followed by a plateau phase, due in part to I_h channels which are activated by hyperpolarization and depolarize the rod membrane potential [48]. Depolarization of the rod membrane potential would restore transmitter release from rods, and this would repolarize the ON bipolar cell, consistent with the observed behavior during steady illumination. However, several lines of evidence suggest that the transient nature of the ON bipolar light response cannot be attributed to a presynaptic effect alone. First, the bipolar cell response is transient at light intensities that produce a sustained response in rods [49]. Second, transient responses are still present when the presynaptic rod input is bypassed completely using a pharmacological approach [44]. Third, transient responses are largely eliminated under conditions when Ca^{2+} is tightly and rapidly buffered with BAPTA [50–52]. In mammalian retina, the effect of Ca^{2+} is rapid, reducing the light response on the order of milliseconds [50], while the effect is somewhat slower in lower vertebrates [51, 52].

The TRPM1 channel is itself permeable to Ca^{2+} [38, 44, 53], and so influx of this cation through the channel could act to signal that the channel has opened in response to light. In principle, Ca^{2+} could restore the membrane potential to near dark levels through the activation of voltage and Ca^{2+}-dependent potassium or chloride channels. Such a mechanism would be independent of an effect on the TRPM1 channel itself. However, several lines of evidence argue against this. The effect persists under voltage clamp, and at a range of holding potentials [50–52]. In addition, it is unaffected when Cs^{+} is substituted for K^{+} in the internal recording solution, or when the Cl^{-} concentration is varied (unpublished observations). It seems more likely that Ca^{2+} acts directly on the mGluR6 cascade to reduce the amplitude of the TRPM1 current. We have referred to this Ca^{2+}-dependent process as desensitization, as we view it to be functionally analogous to the desensitization of ligand-gated channels during agonist binding.

The dependence of desensitization on extracellular Ca^{2+} is illustrated in Fig. 7.1. Here, an ON bipolar cell from the salamander retina is voltage clamped at + 40 mV. The cell is bathed in a solution containing 1 mM glutamate, delivered through an apparatus that allows for rapid local solution change, and so the TRPM1 current is suppressed. The glutamate solution bathing the cell is then rapidly exchanged for a solution containing the mGluR6 antagonist cyclopropyl-4-phosphonophenylglycine (CPPG), and this activates TRPM1 current, which at this voltage is outward. Note that there is no change in the amplitude of the current with time (i.e., no desensitization). However, when the voltage is stepped to a negative voltage (−20 mV in the top right panel), the TRPM1 current becomes inward and then decays exponentially to a plateau value (black trace). This experiment is consistent with the idea that the driving force is sufficient to drive Ca^{2+} through the TRPM1 channel and promote desensitization at −20 mV, but not +40 mV. Stepping to more negative voltages produces a larger TRPM1 current and a greater amount of desensitization (Fig. 7.1a, right panels, summarized in Fig. 7.1b). The role of Ca^{2+} is made clear when the same experiments are repeated in nominally Ca^{2+}-free solutions (Fig. 7.1a, gray traces). Because the driving force for Ca^{2+} increases with hyperpolarization, its effect on the steady-state current is correlated with voltage (Fig. 7.1c). The steady state *I–V* relation of the TRPM1 current is linear in the absence of Ca^{2+}, but rectifies outwardly in the presence of physiologically relevant concentrations of extracellular Ca^{2+}.

Desensitization Makes Light Responses More Transient and Prevents Bipolar and Ganglion Cell Saturation

What advantage does desensitization confer to ON bipolar cells? First, it assists in the conversion of the relatively sustained light response generated by photoreceptor activation to a transient response to light in the inner retina. This effect was quantified in my laboratory by recording from a downstream retinal ganglion cell (RGC) and measuring the number of transmitter quanta that were released from the axon terminal of an upstream bipolar cell during a light event. First, a light response was

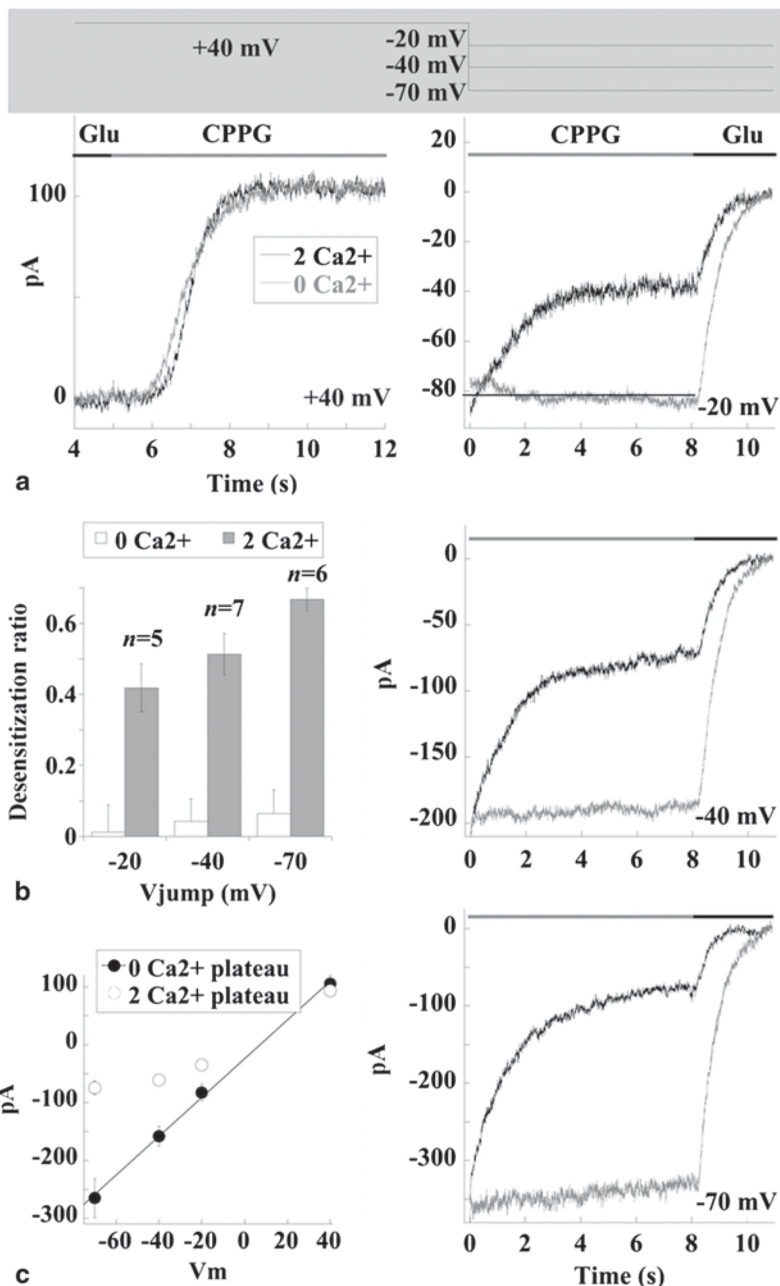

Fig. 7.1 Voltage-dependent properties of the transduction current are conferred by Ca^{2+}. **a** The *shaded area* at the top of the figure shows the voltage protocol for the *four panels* enclosed within the box. On the *left* is the response to the application of cyclopropyl-4-phosphonophenylglycine (*CPPG*) at +40 mV in the presence and absence of Ca^{2+} in the bathing medium. The *three panels* on the *right* illustrate the effect of steps to the indicated voltage, also in the presence and absence of Ca^{2+}. Removing Ca^{2+} essentially eliminates the voltage-dependent decay of the transduction current. **b** Summary of the effects of Ca^{2+} removal on desensitization. **c** *I–V* relation of the plateau phase of the transduction current in 2 mm Ca^{2+} and Ca^{2+}-free solution. Removing Ca^{2+} eliminates the rectification. (Reprinted with permission from [51])

simulated by the application of the mGluR6 antagonist LY341495 and the number of transmitter quanta in the postsynaptic RGC excitatory postsynaptic current (EPSC) was determined. Next, the experiment was repeated using a solution that was nominally Ca^{2+} free, thus blocking Ca^{2+}-dependent desensitization. Blocking desensitization increased quantal release during the simulated light event by nearly fourfold [52]. This is consistent with the idea that desensitization of TRPM1 repolarizes ON bipolar cells sufficiently to close L-type Ca^{2+} channels at the axon terminal, thus reducing transmitter release onto postsynaptic ganglion cells. Desensitization is not the only mechanism for truncation of transmitter release from bipolar cell terminals. Inhibitory feedback from amacrine cells also plays a role, as has been well documented [54–57].

Desensitization of the TRPM1 channel insures that the response to a maintained light event is transient. It differs from adaptation because unlike adaptation, strengthening the stimulus will not result in further signaling by the bipolar cell. Instead, the TRPM1 channel remains in the desensitized state on the order of milliseconds in mouse [50] and up to seconds in cold-blooded vertebrates [52]. This might seem to be counterproductive, preventing bipolar cells from signaling during the time period when TRPM1 channels are recovering from desensitization. However, it is advantageous for several reasons. Synaptic currents generated by the opening of TRPM1 are rather small, and bipolar cells require a high input resistance to be sure that the currents generated at the distal dendrites reliably depolarize the axon terminal at the other end of the cell. If the light stimulus impinging on the presynaptic photoreceptor pool is not homogeneous, but instead covers only a portion of the photoreceptor pool, then strong stimulation of the TRPM1 channels that are postsynaptic to the stimulated photoreceptors could have a shunting effect, lowering the input resistance and reducing the response to stimulation of other regions of the dendritic tree. Such a mechanism would seem to be particularly advantageous for ON bipolar cells that sample from a large number of photoreceptors, such as in the tiger salamander [58].

The same argument can be extended to the ganglion cell. Depending on the type and eccentricity in the retina, ganglion cells can receive input from many ON bipolar cells. Illumination of a portion of the ganglion cell receptive field may result in saturation of ganglion cell spike frequency, rendering the cell incapable of increasing its fire rate should the stimulus increase in size. Desensitization assures that long-lasting light stimuli detected in a subunit of the total ganglion cell receptive field will increase spike frequency only transiently, freeing the ganglion cell to increase spike rate in response to incoming signals from other regions of its receptive field.

Mechanism of Ca^{2+} Desensitization

Many Ca^{2+}-mediated signaling processes involve Ca^{2+}-binding proteins, which contain EF-hand domains that bind to Ca^{2+} ions [59, 60]. Calmodulin (CaM) is a ubiquitous Ca^{2+}-binding protein containing four EF-hand motifs, each with distinct

affinities for Ca^{2+}. One possibility is that CaM binds directly to TRPM1 leading to a conformational change that is the molecular basis of desensitization. Alternatively, CaM might activate a kinase (CaMKII) or phosphatase (calcineurin). However, neither the CaM antagonist calmidazolium nor a CaM inhibitory peptide affected TRPM1 desensitization (Kaur and Nawy, unpublished observations). It should be noted that CaM binds Ca^{2+} with high affinity [61], and it can often be difficult to disrupt this complex using a pharmacological approach [62]. It has been suggested that activation of CaMKII is a requirement [63], and there is a precedent for modulation of desensitization of the vanilloid family of TRP channels, Trpv1 [64], and the N-methyl D-aspartate (NMDA) receptor by CaMKII [65–67], but a lack of effect of CaMKII antagonists on desensitization has also been reported [51] (see the next section for a calcineurin-dependent form of TRPM1 regulation by Ca^{2+}). A pharmacological approach may not allow for a definitive conclusion. Instead, the use of genetic approaches, such as expression of dominant negative forms of CaM that do not transduce Ca^{2+}, may be a better approach [68, 69].

An intriguing possibility is that the sensor for Ca^{2+} desensitization is not located on the TRPM1 channel itself, but at a more distant location. Diffusion of Ca^{2+} through the channel pore and subsequent binding of Ca^{2+} to the channel would require movement of the ion across a very small nanodomain [70–72]. Conversely, if the Ca^{2+} sensor is located at a different site, Ca^{2+} would be required to diffuse across a distance of several microns from the mouth of the channel. Thus, the Ca^{2+} domain would be on the order of microns rather than nanometers [73–76]. For several reasons, the microdomain model is more consistent with the present data. First, small reductions in synaptic glutamate produce TRPM1 currents that do not undergo desensitization [52]. This is true if the observed current is approximately 20 % or less of the maximum evoked current. The lack of desensitization under conditions when only a small fraction of TRPM1 channels open seems useful, as it increases the probability that small signals generated by the opening of such a few number of channels will be passed along to postsynaptic cells. This finding is in conflict with the nanodomain model where desensitization is a property of individual channel and therefore should be observed for all measurable current amplitudes.

Another line of evidence supporting the microdomain model comes from experiments using the Ca^{2+} chelators ethylene glycol tetraacetic acid (EGTA) and 1, 2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA). Both bind Ca^{2+} equally well at equilibrium, but EGTA binds Ca^{2+} ~100-fold more slowly than BAPTA [76, 77]. Thus, if the Ca^{2+} site of action is near the channel, BAPTA is more effective than EGTA at sequestering Ca^{2+}. In contrast to this prediction, BAPTA and EGTA are equally effective at blocking desensitization, supporting the idea that diffusion of Ca^{2+} to the effector is sufficiently far to allow both chelators an opportunity to bind the cation [50–52]. Finally, the time course of recovery from TRPM1 desensitization has been shown to be proportional to the number of TRPM1 channels that open during a synaptic response [52]. A nanodomain model would predict that the rate of recovery from desensitization for a given TRPM1 channel should be independent of the total number of open channels, and instead should be governed by the kinetic properties of the channel itself. Instead, recovery from

desensitization seems to reflect more global changes in Ca^{2+} concentration, perhaps indicating the presence of a Ca^{2+} sensor that is separate from the channel. Finally, desensitization has been reported for rod, but not cone, bipolar cells in the mammalian retina [50]. Whether this is due to differences in the molecular structure of cone and rod TRPM1 or to differences in the upstream cascade is an intriguing and as yet uninvestigated question.

A Separate, Calcineurin-Dependent Form of Ca^{2+} Regulation

There is a second form of Ca^{2+}-dependent regulation of TRPM1 current, well documented in lower vertebrates but not yet established in mammalian retina. The calcineurin-dependent and the independent form of desensitization complement one another. As discussed above, the independent form requires only a single brief period of Ca^{2+} influx through the open channel, on the order of milliseconds in mammals, but the calcineurin-dependent form is most effectively triggered by frequent, brief openings of the TRPM1 channel. In other words, the calcineurin-dependent form performs more of an integrative function, responding to the fraction of time that the channel spends in the open state [44, 78]. Calcineurin-dependent desensitization occurs over a time period of minutes rather than seconds or milliseconds. Perhaps the strongest evidence for the role of calcineurin is the observation that the introduction of constitutively activated, Ca^{2+}-independent calcineurin through the recording pipette can cause desensitization of TRPM1 even when Ca^{2+} is tightly buffered by inclusion of BAPTA in the recording solution, a condition which typically blocks calcineurin-dependent desensitization (Fig. 7.2). There is precedent for this form of desensitization, as at least one type of desensitization of the Trpv1 channel also requires calcineurin [79].

The calcineurin-dependent and calcineurin-independent forms of desensitization operate through different upstream mechanisms, but might possibly converge on the TRPM1 channel through the same downstream mechanism. The calcineurin-dependent form reduces TRPM1 current by ~60 % [78], similar to the calcineurin-independent form [51]. However, the possibility that the two forms occlude one another has not been formally tested.

Modulation of the mGluR6 Cascade: cGMP

cGMP and Gating of TRPM1

The cyclic nucleotide, cGMP, strongly potentiates TRPM1 currents. Intracellular perfusion of cGMP through a recording pipette or via bath application of membrane-permeant analogs was found to cause a robust increase in TRPM1 current [26, 80]. This was initially interpreted as evidence that cGMP gated the TRPM1

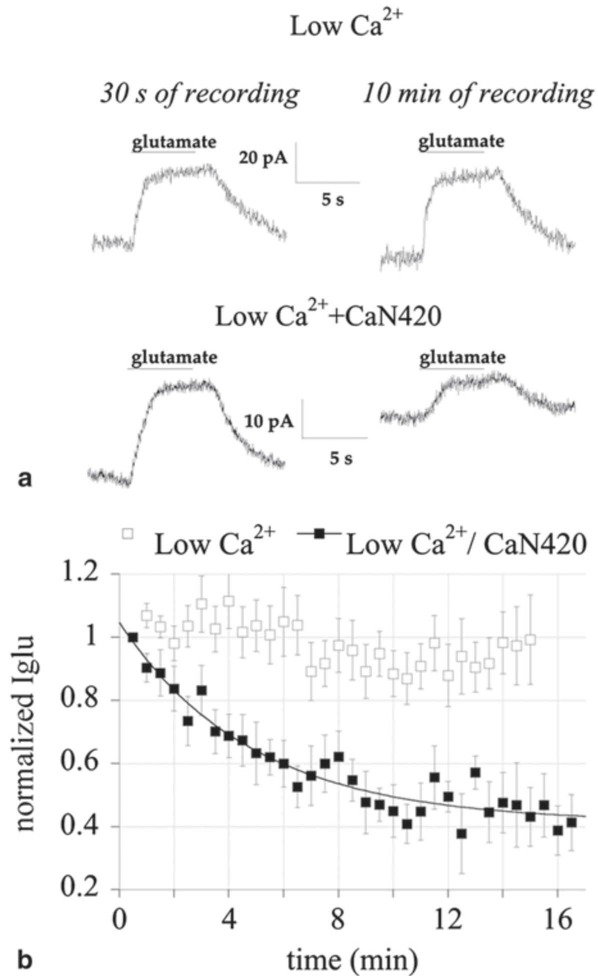

Fig. 7.2 A Ca^{2+}-independent form of calcineurin is sufficient to induce depression in the absence of Ca^{2+}. **a** *Top panel,* example of glutamate-elicited currents recorded from an ON bipolar cell with a pipette containing 20 mM BAPTA (low Ca^{2+} solution). The amplitude of the response remained essentially unchanged over time. *Bottom panel,* example of glutamate-elicited currents recorded from another ON bipolar cell with a pipette containing the low Ca^{2+} solution and 100 nM CaN420. The response was depressed, with a concomitant shift in the baseline, suggesting that CaN420 might downregulate the cation current, as has been proposed for Ca^{2+}. **b** Summary and comparison of the effect on I_{glu} of the low Ca^{2+} solution alone (*open symbols,* $n=10$), and with CaN420 (*closed symbols,* $n=13$). (Reprinted with permission from [78])

channel open. It was hypothesized that endogenous levels of cGMP were sufficient to open only a few of the synaptic channels, serving to reduce noise and maintain a high input resistance, much as had been shown for cGMP and cyclic nucleotide-gated channels in photoreceptors. Introduction of a high concentration of cGMP through the recording pipette would lead to the opening of more synaptic channels than would open under physiological conditions, thus giving the appearance of potentiation. Further, it was proposed that the mGluR6 cascade closed the synaptic channel by decreasing cGMP levels, perhaps by activation of a cGMP-specific phosphodiesterase [26]. The same interpretation was reached by a second, independent group [80].

The idea that the transduction cascade in bipolar cells was essentially the same as the photoreceptor cascade was appealing by virtue of its simplicity, if nothing else. However, several lines of evidence came to light that were inconsistent with

such a model. First, the cGMP-gated channel expressed in rods was absent in the dendrites of ON bipolar cells, as judged by the lack of staining of bipolar cell dendrites using an antibody that detected cGMP channels in rod outer segments [81]. Second, an essential requirement for the cGMP model in its simplest form is that cGMP hydrolysis is required to allow for the current to be shut OFF by glutamate. However, the application of glutamate was found to strongly suppress the current even when highly hydrolysis-resistant forms of cGMP were dialyzed into the cell [28]. Nevertheless, the idea that cGMP was essential for channel gating stubbornly remained in the ON bipolar cell literature, in part because a more appealing model was not rapidly forthcoming.

cGMP Increases Signal Amplification in the mGluR6 Cascade

The initial experiments on cGMP and bipolar cells were conducted in lower vertebrates, but later were expanded to mouse. Two observations were made that provided insight into the mechanism of potentiation. First, the effect of cGMP on TRPM1 required cGMP-dependent kinase. Second, potentiation was only observed on submaximal TRPM1 currents [82]. The second finding, in particular, is intriguing because it implies that phosphorylation of TRPM1, or perhaps another element in the cascade, increases the efficiency of coupling to the mGluR6 cascade. Small perturbations in glutamate release, brought about by absorption of single photons in the rod pool, were highly magnified by the addition of exogenous cGMP. In a series of experiments performed in my laboratory, we found a strong correlation between the magnitude of TRPM1 current and the amount of potentiation produced by cGMP application. In some cases, a brief application of mGluR6 antagonist displaced glutamate from only a few mGluR6 receptors and was not sufficient to open any TRPM1 channels [82]. However, after dialysis with cGMP, the same stimulus resulted in a robust activation of the cascade and opening of TRPM1 channels. Similar effects of cGMP on the mGluR6 cascade have been observed in cat and fish retinas [83, 84].

The Effects of cGMP at ON Bipolar Cells Is Retained in Downstream AII Amacrine Cells

In the mammalian retina, rod bipolar cells are presynaptic to AII amacrine cells rather than ganglion cells [85]. Experiments using paired recordings have demonstrated highly coordinated, multivesicular release of synaptic transmitter from bipolar to amacrine cell when the bipolar cell membrane potential is instantaneously depolarized by the application of a voltage step [86, 87]. But light responses generate depolarizing responses in rod bipolar cells that are both slower and smaller [88–90] than typical voltage steps. Both the amplitude and rise time of the bipolar cell light response impact the resulting AII EPSC. For example, maintaining a constant am-

plitude of 20 mV, but varying the rise time of the bipolar cell depolarization from an instantaneous voltage step to a ramp that lasts 100 ms, decreases the amplitude of the AII EPSC by 50 % and changes the shape from a single peak representing well-orchestrated transmitter release to a series of randomly distributed peaks [91].

Modulation of the mGluR6 cascade by cGMP not only potentiates the peak of the TRPM1 current, but importantly increases the rate of rise as well. This is illustrated in an experiment from my laboratory in which a rod bipolar cell was recorded in current clamp and depolarized by the application of an mGluR6 antagonist in a background of L-AP4, while a downstream AII amacrine cell was voltage clamped in order to monitor the effect of cGMP on the EPSC (Fig. 7.3). Application of CPPG depolarized the rod bipolar cell and elicited an EPSC in the downstream AII amacrine cell. By changing the duration of the CPPG "puff," the size of the bipolar cell depolarization and the resulting postsynaptic EPSC could be varied. Next, a membrane-permeant cGMP analog was applied to the bath and the experiment was repeated. cGMP increased the depolarization rate of the bipolar cell, strongly potentiating the EPSC of the AII amacrine cell.

Historically, studies of cGMP effects in ON bipolar cells have relied on the addition of exogenous cGMP to produce these effects, the implication being that endogenous cGMP levels are insufficient to generate potentiation of TRPM1 currents and downstream AII EPSCs. In the intact retina, changes in cGMP levels are most likely mediated by the second messenger nitric oxide (NO). Nitric oxide is synthesized by nitric oxide synthase, most likely the neuronal form (nNOS) which predominates in the retina [92, 93]. ON bipolar cells express a NO-activated guanylyl cyclase [94–96], the only known receptor for NO, and stimulation with NO or its analogs upregulates cGMP levels in ON bipolar cells [97–99] and potentiates TRPM1 currents [82]. Thus, the machinery for elevating cGMP is present in ON bipolar cells. One explanation for the apparently low concentration of endogenous cGMP in bipolar cells may be that the levels of endogenous NO are compromised in a slice preparation. A more intriguing possibility is that NO production is promoted by specific patterns of illumination [100, 101] and would be absent in retina preparations that are maintained under conditions of continuous illumination. Since nNOS is distributed throughout the retina, including bipolar cells, amacrine cells, and nearby photoreceptors [92], the issue of whether production of cGMP is autocrine or paracrine is unresolved and will require further study.

Modulation of the mGluR6 Cascade: PKC and DAG

Finally, there is evidence that activation of PKCα through the canonical phospholipase C (PLC) pathway results in potentiation of TRPM1 [45]. Interestingly, PKC seems to exert its actions by relieving the inhibition of TRPM1 by intracellular Mg^{2+} [45], as activation of PKCα with the DAG analog 1-oleoyl-2-acetyl-sn-glycerol (OAG) has no effect at intracellular Mg^{2+} concentrations below 0.5 mM. The trigger for activation of this pathway appears to be the binding of synaptic glu-

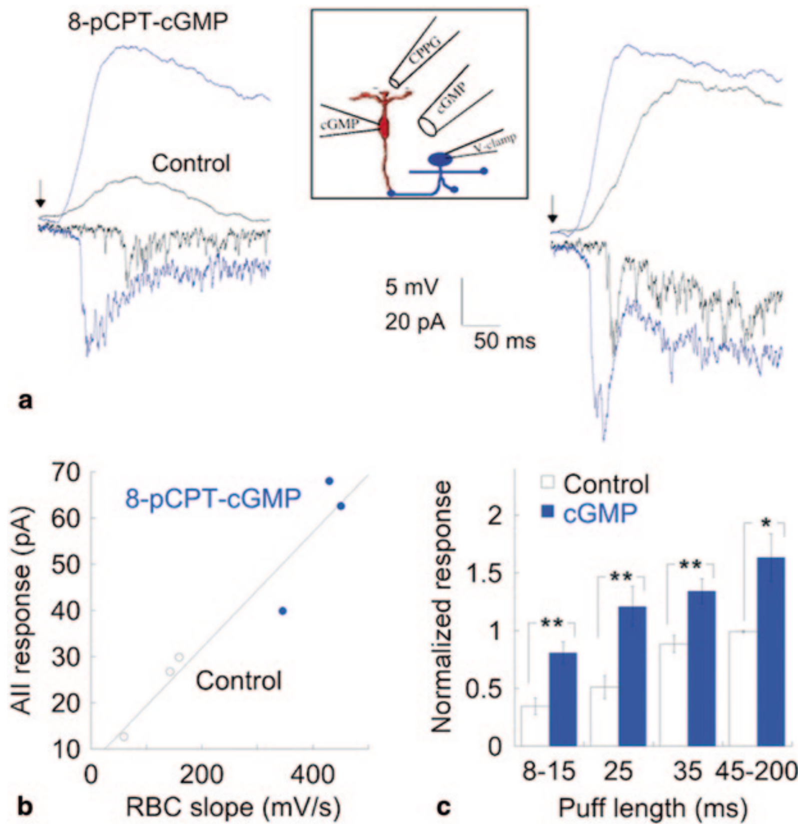

Fig. 7.3 Potentiation of rod bipolar cell (*RBC*) responses by cyclic guanosine monophosphate (*cGMP*) that in turn potentiates AII EPSCs. **a** Averaged response of an RBC–AII pair to an 8-ms puff (*left*), or 50-ms puff (*right*) of cyclopropyl-4-phosphonophenylglycine (*CPPG*) following break-in (*black trace*) and after the local application of 1 mM 8-pCPT-cGMP (*blue trace*). *Inset,* experimental protocol; in some experiments, *cGMP* was dialyzed directly into individual *RBCs,* while in others, such as the one shown here, it was added in a membrane-permeant form. **b** *Left,* effects of *cGMP* on the rate of depolarization of the *RBC* and the peak AII response from the pair shown in **a**. Puff lengths were 8, 35, and 200 ms. *Open symbols* are before and *filled symbols* are after 8-pCPT-cGMP application, respectively. **c** Summary of the effects of *cGMP* on AII response amplitude as a function of puff duration. Data from bath and intracellular application of *cGMP* have been pooled, and puff lengths have been binned as labeled. $n=8$ for all puff length bins except for 25 ms ($n=6$). (Reprinted with permission from [91])

tamate to Group I metabotropic receptors that have been shown to be expressed specifically in rod, but not cone, bipolar cells, and whose activation elicits Ca^{2+} release from stores in the vicinity of the distal dendrites [102]. Interestingly, PKCα itself is expressed only by rod, and not by cone, bipolar cells [103], and in fact the relief of Mg^{2+} block by PKC activation is observed only in rod bipolar cells [45]. Inhibition of Group I mGluR receptors with specific antagonists, or presentation of dim background illumination, which has a suppressive effect on glutamate re-

lease from photoreceptors, both block potentiation by PKCα (Rampino and Nawy, unpublished observations). Unlike the effect of cGMP described above, PKCα potentiates TRPM1 current at every light intensity, consistent with the idea that it acts directly on the channel to relieve Mg^{2+} inhibition, rather than to modulate coupling to mGluR6 cascade.

Measurements of ON bipolar cell function in a PKCα$^{-/-}$ mouse line obtained by monitoring the b-wave of the electroretinogram (ERG) do not reveal the expected decrease in amplitude, but instead argue for the improper, delayed shutOFF of the TRPM1 current after the termination of the light response [104]. Conversely, patch clamp recordings from rod bipolar cells of the same mouse line reveal a diminished TRPM1 current, which cannot be rescued by the addition of DAG analogs [45]. It should be noted that the b-wave does not directly reflect the behavior of the dendritic TRPM1 current, but rather it takes into account all inputs that modulate the membrane potential of bipolar cells, including inhibitory feedback. In fact, measurements of inhibitory feedback onto rod bipolar cell terminals support the idea that feedback is greatly reduced in the PKCα-knockout mouse [45], perhaps explaining the discrepancy between the patch clamp and ERG studies.

Concluding Remarks

The synapse between photoreceptors and ON bipolar cell has long fascinated those of us in this field. The problem was to create a sign inversion at a synapse where there should not be one, to generate an inhibitory synapse using an excitatory transmitter. Evolution left us with an outside-the-box solution to this problem. At each end of the synaptic pathway, one finds a glutamate receptor and an ion channel that are expressed in only one type of cell in the nervous system. In the middle, a ubiquitously expressed G protein whose inactivation, a necessity for generating a light response, is apparently more tightly regulated than its activation. At first glance, this would appear to be the Rube Goldberg of synaptic mechanisms, as one could imagine more familiar and seemingly simple solutions to solve this problem. But the use of a channel-closing mechanism allows for the highest possible input resistance (and therefore synaptic gain) in the dark, while the choice of a membrane-delimited pathway ensures high fidelity and speed.

Although the photoreceptor–ON bipolar cell synapse is the first in the visual system, even at this early stage of processing, there is a tremendous capacity for synaptic plasticity. Synaptic gain is reduced by Ca^{2+} through at least two separate pathways and time scales, and is an important mechanism for restoring ON bipolar cell membrane potential and input resistance to near dark levels during maintained illumination. Conversely, gain can be increased by pathways involving NO and mGluR1/5 and their canonical signaling pathways. This is a highly unusual, perhaps unique synapse, and the last decade of research has elucidated novel forms of synaptic plasticity that have coevolved to meet its needs. Undoubtedly, there are more surprises waiting to be discovered.

Acknowledgments Supported by funding from the NEI (EY010254) and by an unrestricted grant from the RPB foundation.

References

1. Kaneko A (1970) Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. J Physiol 207(3):623–633
2. Werblin FS, Dowling JE (1969) Organization of the retina of the mudpuppy, Necturus maculosus. II. Intracellular recording. J Neurophysiol 32(3):339–355
3. Fain GL (1975) Interactions of rod and cone signals in the mudpuppy retina. J Physiol 252(3):735–769
4. Stell WK (1967) The structure and relationships of horizontal cells and photoreceptor-bipolar synaptic complexes in goldfish retina. Am J Anat 121(2):401–423
5. Stell WK, Ishida AT, Lightfoot DO (1977) Structural basis for on-and OFF-center responses in retinal bipolar cells. Science 198(4323):1269–1271
6. Ishida AT, Stell WK, Lightfoot DO (1980) Rod and cone inputs to bipolar cells in goldfish retina. J Comp Neurol 191(3):315–335
7. Cajal SRy (1892) La retine des vertabres. Cellule 9:121–225
8. Scholes JH (1975) Colour receptors, and their synaptic connexions, in the retina of a cyprinid fish. Philos Trans R Soc Lond B Biol Sci 270(902):61–118
9. Parthe V (1972) Horizontal, bipolar and oligopolar cells in the teleost retina. Vision Res 12(3):395–406
10. Saito T, Kondo H, Toyoda JI (1979) Ionic mechanisms of two types of on-center bipolar cells in the carp retina. I. The responses to central illumination. J Gen Physiol 73(1):73–90
11. Kondo H, Toyoda J-I (1980) Dual effect of glutamate and aspartate on the on-center bipolar cell in the carp retina. Brain Res 199(1):240–243
12. Saito T, Kujiraoka T (1982) Physiological and morphological identification of two types of on-center bipolar cells in the carp retina. J Comp Neurol 205(2):161–170
13. Saito T, Kondo H, Toyoda J-i (1978) Rod and cone signals in the on-center bipolar cell: their different ionic mechanisms. Vision Res 18(5):591–595
14. Saito T, Kondo H (1978) Ionic mechanisms underlying the center and surround responses of on-center bipolar cells in the carp retina. Sens Processes 2(4):350–358
15. Saito T, Kaneko A (1983) Ionic mechanisms underlying the responses of OFF-center bipolar cells in the carp retina. I. Studies on responses evoked by light. J Gen Physiol 81(4):589–601
16. Kaneko A, Shimazaki H (1975) Effects of external ions on the synaptic transmission from photorecptors to horizontal cells in the carp retina. J Physiol 252(2):509–522
17. Slaughter MM, Miller RF (1981) 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. Science 211(4478):182–185
18. Shiells RA, Falk G, Naghshineh S (1981) Action of glutamate and aspartate analogues on rod horizontal and bipolar cells. Nature 294(5841):592–594
19. Schiller PH, Sandell JH, Maunsell JH (1986) Functions of the ON and OFF channels of the visual system. Nature 322(6082):824–825
20. Nawy S, Copenhagen DR (1987) Multiple classes of glutamate receptor on depolarizing bipolar cells in retina. Nature 325(6099):56–58
21. Grant GB, Dowling JE (1995) A glutamate-activated chloride current in cone-driven ON bipolar cells of the white perch retina. J Neurosci 15(5 Pt 2):3852–3862
22. Grant GB, Dowling JE (1996) ON bipolar cell responses in the teleost retina are generated by two distinct mechanisms. J Neurophysiol 76(6):3842–3849
23. Adams PR, Brown DA (1982) Synaptic inhibition of the M-current: slow excitatory postsynaptic potential mechanism in bullfrog sympathetic neurones. J Physiol 332(1):263–272
24. Brown D (1988) M-currents: an update. Trends Neurosci 11(7):294–299
25. Nawy S, Jahr CE (1990) Time-dependent reduction of glutamate current in retinal bipolar cells. Neurosci Lett 108(3):279–283

26. Nawy S, Jahr CE (1990) Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. Nature 346(6281):269–271
27. Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N et al (1993) Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. J Biol Chem 268(16):11868–11873
28. Nawy S (1999) The metabotropic receptor mGluR6 may signal through G_o, but not phosphodiesterase, in retinal bipolar cells. J Neurosci 19(8):2938–2944
29. Dhingra A, Lyubarsky A, Jiang M, Pugh EN, Birnbaumer L, Sterling P et al (2000) The light response of ON bipolar neurons requires G[alpha]o. J Neurosci 20(24):9053–9058
30. Sandmeyer LS, Breaux CB, Archer S, Grahn BH (2007) Clinical and electroretinographic characteristics of congenital stationary night blindness in the Appaloosa and the association with the leopard complex. Vet Ophthalmol 10(6):368–375
31. Bellone RR, Brooks SA, Sandmeyer L, Murphy BA, Forsyth G, Archer S et al (2008) Differential gene expression of TRPM1, the potential cause of congenital stationary night blindness and coat spotting patterns (LP) in the Appaloosa horse (Equus caballus). Genetics 179(4):1861–1870
32. Duncan LM, Deeds J, Hunter J, Shao J, Holmgren LM, Woolf EA et al (1998) Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. Cancer Res 58(7):1515–1520
33. Duncan LM, Deeds J, Cronin FE, Donovan M, Sober AJ, Kauffman M et al (2001) Melastatin expression and prognosis in cutaneous malignant melanoma. J Clin Oncol 19(2):568–576
34. Oancea E, Wicks N (2011) TRPM1: new trends for an old TRP. In: Islam MS (ed) Transient receptor potential channels. Advances in experimental medicine and biology, vol 704. Springer, Netherlands, pp 135–145
35. Shen Y, Heimel JA, Kamermans M, Peachey NS, Gregg RG, Nawy S (2009) A transient receptor potential-like channel mediates synaptic transmission in rod bipolar cells. J Neurosci 29(19):6088–6093
36. Koike C, Obara T, Uriu Y, Numata T, Sanuki R, Miyata K et al (2010) TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. Proc Natl Acad Sci U S A 107(1):332–337
37. Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS, Duvoisin RM et al (2009) TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proc Natl Acad Sci U S A 106(45):19174–19178
38. Shen Y, Rampino MAF, Carroll RC, Nawy S (2012) G-protein-mediated inhibition of the Trp channel TRPM1 requires the Gβγ dimer. Proc Natl Acad Sci U S A 109(22):8752–8757
39. Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NM, Hille B (1985) GTP-binding proteins couple cardiac muscarinic receptors to a K channel. Nature 317(6037):536–538
40. Breitwieser GE, Szabo G (1985) Uncoupling of cardiac muscarinic and beta-adrenergic receptors from ion channels by a guanine nucleotide analogue. Nature 317(6037):538–540
41. Wickman KD, Iniguez-Lluhl JA, Davenport PA, Taussig R, Krapivinsky GB, Linder ME et al (1994) Recombinant G-protein beta gamma-subunits activate the muscarinic-gated atrial potassium channel. Nature 368(6468):255–257
42. Holz GGt, Rane SG, Dunlap K (1986) GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. Nature 319(6055):670–672
43. Scott RH, Dolphin AC (1986) Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen-mediated inhibition. Neurosci Lett. 69(1):59–64
44. Nawy S (2000) Regulation of the ON bipolar cell mGluR6 pathway by Ca2+. J Neurosci 20(12):4471–4479
45. Rampino MAF, Nawy SA (2011) Relief of Mg2+-dependent inhibition of TRPM1 by PKCα at the rod bipolar cell synapse. J Neurosci 31(38):13596–13603
46. Oancea E, Vriens J, Brauchi S, Jun J, Splawski I, Clapham DE (2009) TRPM1 forms ion channels associated with melanin content in melanocytes. Sci Signal 2(70):ra21
47. Koike C, Numata T, Ueda H, Mori Y, Furukawa T (2010) TRPM1: a vertebrate TRP channel responsible for retinal ON bipolar function. Cell Calcium 48(2–3):95–101

48. Fain GL, Quandt FN, Bastian BL, Gerschenfeld HM (1978) Contribution of a caesium-sensitive conductance increase to the rod photoresponse. Nature 272(5652):466–469
49. Shiells RA, Falk G (1999) Ca(2+)-induced light adaptation in retinal ON-bipolar cells. Keio J Med 48(3):140–146
50. Berntson A, Smith RG, Taylor WR (2004) Postsynaptic calcium feedback between rods and rod bipolar cells in the mouse retina. Vis Neurosci 21(6):913–924
51. Nawy S (2004) Desensitization of the mGluR6 transduction current in tiger salamander ON bipolar cells. J Physiol 558(Pt 1):137–146
52. Kaur T, Nawy S (2012) Characterization of TRPM1 desensitization in ON bipolar cells and its role in downstream signalling. J Physiol 590(1):179–192
53. Xu XZS, Moebius F, Gill DL, Montell C (2001) Regulation of melastatin, a TRP-related protein, through interaction with a cytoplasmic isoform. Proc Natl Acad Sci U S A 98(19):10692–10697
54. Roska B, Nemeth E, Werblin FS (1998) Response to change is facilitated by a three-neuron disinhibitory pathway in the tiger salamander retina. J Neurosci 18(9):3451–3459
55. Dong C-J, Werblin FS (1998) Temporal contrast enhancement via GABAC feedback at bipolar terminals in the tiger salamander retina. J Neurophysiol 79(4):2171–2180
56. Lukasiewicz P, Werblin F (1994) A novel GABA receptor modulates synaptic transmission from bipolar to ganglion and amacrine cells in the tiger salamander retina. J Neurosci 14(3):1213–1223
57. Zhang J, Slaughter MM (1995) Preferential suppression of the ON pathway by GABAC receptors in the amphibian retina. J Neurophysiol 74(4):1583–1592
58. Lasansky A (1978) Contacts between receptors and electrophysiologically identified neurones in the retina of the larval tiger salamander. J Physiol 285:531–542
59. Clapham DE (2007) Calcium signaling. Cell 131(6):1047–1058
60. Nakayama S, Kretsinger RH (1994) Evolution of the EF-hand family of proteins. Annu Rev Biophys Biomol Struct 23:473–507
61. Gilli R, Lafitte D, Lopez C, KilhOFFer M, Makarov A, Briand C et al (1998) Thermodynamic analysis of calcium and magnesium binding to calmodulin. Biochemistry 37(16):5450–5456
62. Levitan IB (1999) It is calmodulin after all! Mediator of the calcium modulation of multiple ion channels. Neuron 22(4):645–648
63. Shiells RA, Falk G (2000) Activation of Ca2+-calmodulin kinase II induces desensitization by background light in dogfish retinal 'on' bipolar cells. J Physiol 528 Pt 2(1):327–338
64. Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M (2003) Structural determinant of TRPV1 desensitization interacts with calmodulin. Proc Natl Acad Sci U S A 100(13):8002–8006
65. Ikura M (1996) Calcium binding and conformational response in EF-hand proteins. Trends Biochem Sci 21(1):14–17
66. Sessoms-Sikes S, Honse Y, Lovinger DM, Colbran RJ (2005) CaMKIIalpha enhances the desensitization of NR2B-containing NMDA receptors by an autophosphorylation-dependent mechanism. Mol Cell Neurosci 29(1):139–147
67. Tong G, Shepherd D, Jahr CE (1995) Synaptic desensitization of NMDA receptors by calcineurin. Science 267(5203):1510–1512
68. Peterson BZ, DeMaria CD, Yue DT (1999) Calmodulin is the Ca2+ sensor for Ca2+-dependent inactivation of L-type calcium channels. Neuron 22(3):549–558
69. Qin N, Olcese R, Bransby M, Lin T, Birnbaumer L (1999) Ca2+-induced inhibition of the cardiac Ca2+ channel depends on calmodulin. Proc Natl Acad Sci U S A 96(5):2435–2438
70. Simon SM, Llinas RR (1985) Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. Biophys J 48(3):485–498
71. Chad JE, Eckert R (1984) Calcium domains associated with individual channels can account for anomalous voltage relations of CA-dependent responses. Biophys J 45(5):993–999
72. Oheim M, KirchhOFF F, Stuhmer W (2006) Calcium microdomains in regulated exocytosis. Cell Calcium 40(5–6):423–439

73. Neher E (1998) Vesicle pools and Ca2+ microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 20(3):389–399
74. Parekh AB (2008) Ca2+microdomains near plasma membrane Ca2+channels: impact on cell function. J Physiol 586(13):3043–3054
75. Llinas R, Sugimori M, Silver RB (1995) Time resolved calcium microdomains and synaptic transmission. J Physiol Paris 89(2):77–81
76. Neher E (1998) Usefulness and limitations of linear approximations to the understanding of Ca++ signals. Cell Calcium 24(5–6):345–357
77. Marty A, Neher E (1985) Potassium channels in cultured bovine adrenal chromaffin cells. J Physiol 367(1):117–141
78. Snellman J, Nawy S (2002) Regulation of the retinal bipolar cell mGluR6 pathway by calcineurin. J Neurophysiol 88(3):1088–1096
79. Mohapatra DP, Nau C (2005) Regulation of Ca2+-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J Biol Chem 280(14):13424–13432
80. Shiells RA, Falk G (1990) Glutamate receptors of rod bipolar cells are linked to a cyclic GMP cascade via a G-protein. ProcBiol Sci 242(1304):91–94
81. Wassle H, Grunert U, Cook NJ, Molday RS (1992) The cGMP-gated channel of rod outer segments is not localized in bipolar cells of the mammalian retina. Neurosci Lett 134(2):199–202
82. Snellman J, Nawy S (2004) cGMP-dependent kinase regulates response sensitivity of the mouse on bipolar cell. J Neurosci 24(29):6621–6628
83. de la Villa P, Kurahashi T, Kaneko A (1995) L-glutamate-induced responses and cGMP-activated channels in three subtypes of retinal bipolar cells dissociated from the cat. J Neurosci 15(5 Pt 1):3571–3582
84. Shiells RA, Falk G (2002) Potentiation of ‘on’ bipolar cell flash responses by dim background light and cGMP in dogfish retinal slices. J Physiol 542(Pt 1):211–220
85. Bloomfield SA, Dacheux RF (2001) Rod vision: pathways and processing in the mammalian retina. Prog Retin Eye Res 20(3):351–384
86. Singer JH, Lassova L, Vardi N, Diamond JS (2004) Coordinated multivesicular release at a mammalian ribbon synapse. Nat Neurosci 7(8):826–833
87. Singer JH, Diamond JS (2003) Sustained Ca2+ entry elicits transient postsynaptic currents at a retinal ribbon synapse. J Neurosci 23(34):10923–10933
88. Sampath AP, Rieke F (2004) Selective transmission of single photon responses by saturation at the rod-to-rod bipolar synapse. Neuron 41(3):431–443
89. Field GD, Rieke F (2002) Nonlinear signal transfer from mouse rods to bipolar cells and implications for visual sensitivity. Neuron 34(5):773–785
90. Dunn FA, Doan T, Sampath AP, Rieke F (2006) Controlling the gain of rod-mediated signals in the mammalian retina. J Neurosci 26(15):3959–3970
91. Snellman J, Zenisek D, Nawy S (2009) Switching between transient and sustained signalling at the rod bipolar-AII amacrine cell synapse of the mouse retina. J Physiol 587(11):2443–2455
92. Vielma AH, Retamal MA, Schmachtenberg O (2012) Nitric oxide signaling in the retina: what have we learned in two decades? Brain Res 1430:112–125
93. Dawson TM, Bredt DS, Fotuhi M, Hwang PM, Snyder SH (1991) Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc Natl Acad Sci U S A 88(17):7797–7801
94. Ahmad I, Barnstable CJ (1993) Differential laminar expression of particulate and soluble guanylate cyclase genes in rat retina. Exp Eye Res 56(1):51–62
95. Haberecht MF, Schmidt HH, Mills SL, Massey SC, Nakane M, Redburn-Johnson DA (1998) Localization of nitric oxide synthase, NADPH diaphorase and soluble guanylyl cyclase in adult rabbit retina. Vis Neurosci 15(5):881–890
96. Spreca A, Giambanco I, Rambotti MG (1999) Ultracytochemical study of guanylate cyclases A and B in light- and dark-adapted retinas. Histochem J 31(7):477–483
97. Koistinaho J, Swanson RA, de Vente J, Sagar SM (1993) NADPH-diaphorase (nitric oxide synthase)-reactive amacrine cells of rabbit retina: putative target cells and stimulation by light. Neuroscience 57(3):587–597

98. Baldridge WH, Fischer AJ (2001) Nitric oxide donor stimulated increase of cyclic GMP in the goldfish retina. Vis Neurosci 18(6):849–856
99. Gotzes S, de Vente J, Muller F (1998) Nitric oxide modulates cGMP levels in neurons of the inner and outer retina in opposite ways. Vis Neurosci 15(5):945–955
100. Eldred WD, Blute TA (2005) Imaging of nitric oxide in the retina. Vis Res 45(28):3469–3486
101. Giove TJ, Deshpande MM, Eldred WD (2009) Identification of alternate transcripts of neuronal nitric oxide synthase in the mouse retina. J Neurosci Res 87(14):3134–3142
102. Koulen P, Kuhn R, Wassle H, Brandstatter JH (1997) Group I metabotropic glutamate receptors mGluR1alpha and mGluR5a: localization in both synaptic layers of the rat retina. J Neurosci 17(6):2200–2211
103. Greferath U, Grunert U, Wassle H (1990) Rod bipolar cells in the mammalian retina show protein kinase C-like immunoreactivity. J Comp Neurol 301(3):433–442
104. Ruether K, Feigenspan A, Pirngruber J, Leitges M, Baehr W, Strauss O (2010) PKCα is essential for the proper activation and termination of rod bipolar cell response. Invest Ophthalmol Vis Sci 51(11):6051–6058

Chapter 8
The Role of Dopamine in Fine-Tuning Cone- and Rod-Driven Vision

Rolf Herrmann and Vadim Y. Arshavsky

Abstract Dopamine, one of the major neuromodulators in the retina, acts through dopamine D1, D2, and D4 G-protein-coupled receptors localized to many types of retinal neurons. This expansive expression pattern allows dopamine to regulate visual processing at different cellular sites of the retinal circuitry. One of the most extensively studied functions of dopamine is its role in mediating the shift from rod-dominant to cone-dominant vision at the transition from night to daylight. However, dopamine is also critical for the regulation of the rod-driven circuitry. Recent studies in the mouse demonstrated that dopamine enhances the light sensitivity of the rod bipolar cells which mediate vision under dim-to-moderate illumination. In this mechanism, dopamine acts via dopamine D1 receptors and induces a GABAergic input onto rod bipolar cells which evokes a sustained hyperpolarizing chloride current carried by the $GABA_C$ receptor channel. This sensitizing GABAergic input is crucial for increasing the light response amplitudes and extending the operational range of rod bipolar cells. These recent findings expanded the role of dopamine in retinal processing from its well-established function of supporting the transition between rod- and cone-dominant vision to enhancing light responses in the dark or under dim light.

Introduction

Dopamine, acting through its G-protein-coupled receptors (GPCRs), is a major catecholamine neuromodulator in the brain where it regulates a variety of physiological functions, such as locomotor activity, cognition, reward behavior, or emotion [1, 2]. Misbalances in the dopaminergic system can cause dramatic effects which

V. Y. Arshavsky (✉)
Department of Ophthalmology and Pharmacology, Albert Eye Research Institute,
Duke University Medical Center, Room 5012, 2351, Erwin Road, Durham, NC 27710, USA
e-mail: vadim.arshavsky@duke.edu

R. Herrmann
Department of Ophthalmology and Cell Biology, Neurobiology and Anatomy, The Eye Institute,
Medical College of Wisconsin, 925 N. 87th Street, Milwaukee, WI 53226 USA
e-mail: rherrmann@mcw.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_8

often culminate in diseases such as Parkinson's disease, schizophrenia, or Tourette's syndrome [1, 3–5]. Consistent with the key role of dopamine in mediating diverse aspects of brain function, the abuse of drugs which act on dopaminergic neurons (e.g., amphetamine and cocaine) causes severe addictive behavior [6].

Mammalian species have two distinct families of dopamine receptors, the D1 type and the D2 type, both belonging to the rhodopsin-like family of GPCRs (for a detailed review, see [1]). The D1-type family includes the dopamine D1 (D1R) and D5 receptors (D5R), whereas the dopamine D2 (D2R), D3 (D3R), and D4 (D4R) receptors constitute the D2-type family. These two receptor families are functionally distinguished by their effects on the activity of adenylate cyclase. Receptors of the D1 family activate the G protein G_s that stimulates the cyclase activity resulting in the elevation of intracellular cyclic adenosine monophosphate (cAMP) levels. In contrast, activation of D2-family receptors causes inhibition of adenylate cyclase via the G protein G_i and a reduction in cAMP levels. Given the central role of cAMP in mediating diverse cellular functions, dopamine acting on D1- or D2-type receptors can differentially modulate various aspects of cellular signaling.

Studies of the brain demonstrated that, in addition to this conventional role of dopamine regulating the intracellular cAMP, dopamine can initiate other signaling pathways, such as those engaging phospholipase C [7] or arachidonic acid [8]. Furthermore, a large body of recent work revealed that D2 receptors can function through a signaling cascade engaging protein kinase B (Akt), GSK-3 (glycogen synthase kinase 3), and β-arrestin-2 [9]. Growing evidence suggests that the latter mode of cellular signaling underlies pathophysiology of various psychiatric and neurological disorders [10].

Dopamine has also been recognized as an important neuromodulator in the retina. Numerous studies conducted over the past decades on a variety of vertebrate species revealed that dopamine signaling is implicated in various aspects of retinal physiology including dark/light adaptation, gap junction coupling, synaptic transmission, and cell development (reviewed in [11]). Perhaps the most extensively studied aspect of dopamine signaling in the retina is its role in the light adaptation of multiple neurons, i.e., the adjustment of their sensitivity to changes in ambient illumination. Light adaptation is a fundamental function of the retina because it allows our vision to operate over an enormous range of light intensity changes covering more than nine orders of magnitude [12]. Various components of the retinal circuitry contribute to light adaptation, including the rod-driven pathway mediating vision under dim illumination (Fig. 8.1; [13, 14]). The functional role of dopamine in both dark- and light-adapted retina is the major focus of this chapter.

Dopamine in the Retina

In the mammalian retina, dopamine is synthesized by dopaminergic amacrine cells [15]. The cell body of this neuron is localized at the border between the inner nuclear and inner plexiform layers (OFF sublamina 1) [16, 17], where it projects

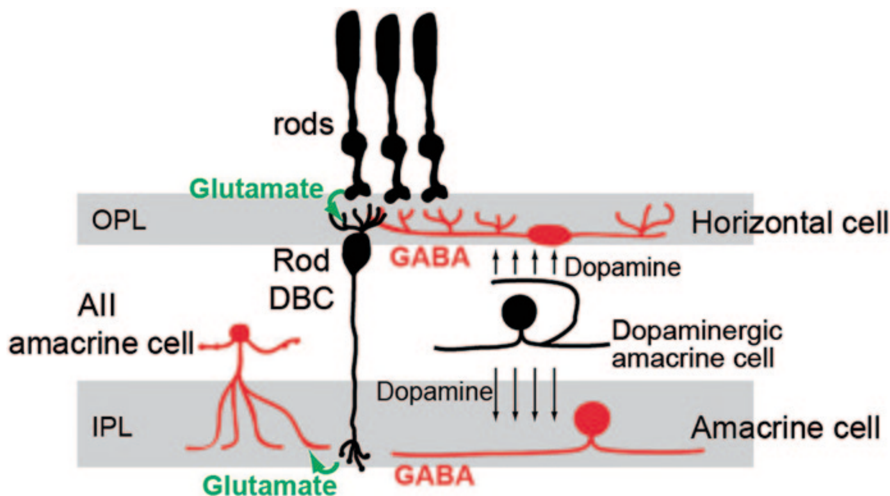

Fig. 8.1 Cartoon illustrating parts of the primary rod-driven pathway in the retina and the major retinal neurons discussed in this chapter. Rod bipolar cells receive glutamatergic inputs from rods in the outer plexiform layer (*OPL*) and provide glutamatergic output onto AII amacrine cells in the inner plexiform layer (*IPL*). AII amacrine cells transmit the signal to cone ON-bipolar cells via electrical synapses (not shown). Dopaminergic amacrine cells release dopamine, which can act on dopamine receptors expressed by horizontal cells in the *OPL* and by amacrine cells in the *IPL*. See text for details. (This and all subsequent figures are reproduced or modified with permission from [20])

axons laterally over large distances up to 500 μm [18]. In addition to this lateral stratification, dopaminergic amacrine cells project multiple processes into the inner plexiform layer. Work on rat and monkey retinas showed that these cells also project axons to the outer plexiform layer where they establish presynaptic contacts with horizontal and bipolar cells [11, 19]. We recently reported a very similar projection pattern for the mouse retina (Fig. 8.2a; [20]). Tyrosine hydroxylase, the enzyme catalyzing the rate-limiting step of dopamine synthesis from tyrosine, is localized throughout the entire dopaminergic amacrine cell, including varicosities and thin dendritic terminals. This distribution suggests that dopamine synthesis (and potentially release) can occur at virtually all locations of this cell. Together with the cell's complex stratification and projection pattern, this allows dopamine to act at multiple cellular sites within the retinal circuitry.

Although the dopaminergic amacrine cells stratify in the OFF sublamina 1, it has been traditionally accepted that these cells do not receive direct inputs from the OFF pathway. Rather, they receive inputs from the ON pathway, which is mediated by a bi-stratifying cone ON bipolar cell as shown in the rabbit retina [21]. The presence of this input from the cone-driven ON pathway indicates that the dopaminergic amacrine cell responds to light increments at intensities that drive cone-mediated visual responses. Based on their light response properties, dopaminergic amacrine cells are surprisingly heterogeneous and can be classified into at least two

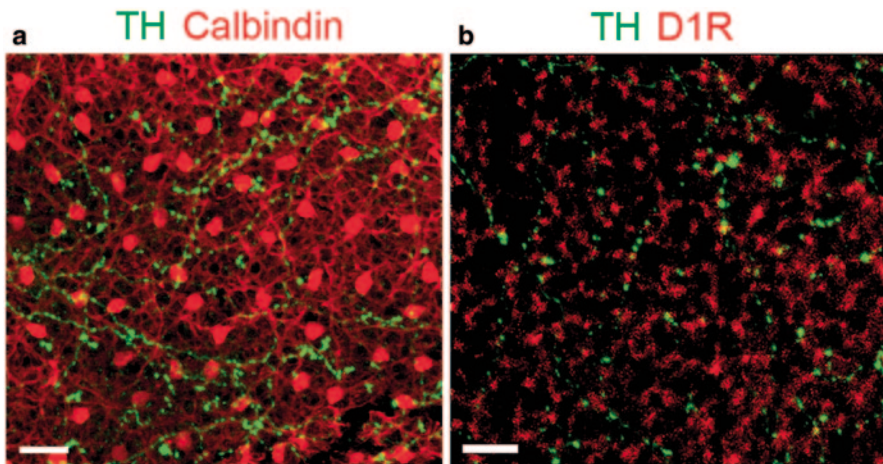

Fig. 8.2 Processes of dopaminergic amacrine cells extend into the horizontal cell layer and are in close proximity to dopamine D1 receptors. **a** Confocal z-stack from a tangential retinal section representing the outer plexiform layer in a wild-type (WT) mouse. The tissue was co-stained for the horizontal cell marker, calbindin (*red*), and the dopaminergic amacrine cell marker, tyrosine hydroxylase (*TH, green*). **b** Confocal z-stack from a tangential WT mouse retinal section representing the outer plexiform layer co-stained for *D1R* (*red*) and *TH* (*green*). Scale bars: 25 µm

distinct classes. The first class displays transient ON responses, whereas the second class responds to light onset in a sustained fashion [22]. Additionally, dopaminergic amacrine cells maintain light-independent, spontaneous electrical activity [18, 22]. Further experiments showed that the ON-transient dopaminergic amacrine cells are excited by ON bipolar cells (consistent with the morphological findings reported by [21]), whereas ON-sustained dopaminergic amacrine cells receive excitatory glutamatergic inputs from intrinsically photosensitive retinal ganglion cells (ipRGCs, [23]). However, a very recent study reported that some dopaminergic amacrine cells respond to dim-light onset with hyperpolarization and a transient suppression of spontaneous spiking activity [24]. This reduction in spontaneous activity to light offset is most likely generated by inhibitory glycinergic inputs from an amacrine cell excited by rod bipolar cells. Therefore, dopaminergic amacrine cells also receive inputs from the rod-driven circuitry.

Even isolated from the retina, dopaminergic amacrine cells display spontaneous spiking activity which induces basal release of dopamine [25]. This is consistent with the findings that the basal level of dopamine release takes place in the dark-adapted retina (see below). With increasing levels of illumination, the dopaminergic amacrine cells increase their spike firing rate and consequently increase dopamine release. In the rabbit retina, steady illumination by bright light causes an elevation of the dopamine release rate by ~66 % relative to the dark [26]. Another study conducted with the fish retina documented an ~twofold increase of dopamine release in response to flickering light [27].

It is commonly thought that dopamine, once released, reaches its receptors extrasynaptically via diffusion, a process often referred to as "volume transmission" [28]. However, it is conceivable that in addition to volume transmission, dopamine could be released at defined synaptic connections. For example, dopaminergic amacrine cells form synapses with AII amacrine cells [29] which were found to express D1-type receptors [30]. Therefore, dopamine could act on its cellular targets both in a synaptic and in an extrasynaptic fashion. Similarly, mouse horizontal cells express dopamine D1 receptors in close proximity to the processes of dopaminergic amacrine cells, which also suggests a synaptic action of dopamine at these sites within the outer plexiform layer (Fig. 8.2b).

While the retina does not express D3R and D5R (the latter was found in the retinal pigment epithelium [31]), D1R, D2R, and D4R have been localized to different types of retinal neurons across different species. D1R has been localized to horizontal cells in mouse and rat retinas using immunohistological techniques [20, 32] and is thought to be expressed in horizontal cells of both cold-blooded vertebrates and primates, based on functional studies (e.g., [33, 34]). D1R is expressed in the inner retina as well. Hayashida et al. reported D1R localization to ganglion cells in the rat retina [35], although this has not been confirmed by others [32]. D1R expression is also documented in several subtypes of cone bipolar and amacrine cells [35]. Accordingly, D1R immunostaining displays a broad pattern in the inner plexiform layer of the mouse retina [20]. A complete set of neuron types in the mouse retina expressing D1R remains to be identified.

The expression pattern of D2R-family receptors, D2R and D4R, appears to be more restrictive and confined predominantly to photoreceptors [36–38]. D2R is also found in dopaminergic amacrine cells [39] where it functions as an autoreceptor for the regulation of dopamine release [25]. However, a recent study detected D4R mRNA in the inner nuclear layer and in ganglion cells of the mouse retina [40], suggesting that the expression pattern and functional role of D4R receptors in the retina might be more complex than presently appreciated.

Regarding the mode of cellular signaling downstream from dopamine receptors, all studies conducted so far in the retina suggested the classical mechanism based on the regulation of intracellular cAMP levels. For example, dopamine-dependent cAMP changes have been shown to underlie photoreceptor or horizontal cell coupling—the adaptation mechanism discussed in detail in the next section. Any involvement of the Akt/GSK3 signaling cascade in the regulation of cellular signaling in retinal neurons remains to be addressed in the future studies.

The Role of Dopamine in Biasing the Retinal Circuitry from Rod- to Cone-Dominant Vision

For a long time, dopamine has been recognized as a neuromodulator that facilitates the switch from rod-dominant to cone-dominant vision at the transition from night to day (there is ample experimental evidence that this mechanism is controlled by

the circadian clock (e.g., [41, 42]). The underlying theme is that dopamine enhances cone-driven responses, while suppressing the flow of rod signals to downstream neurons at different sites of the rod-driven circuitry. This function of dopamine is accomplished via two principle mechanisms: modulation of the coupling state between different classes of retinal neurons (i.e., photoreceptors, horizontal cells, amacrine cells, and ganglion cells) and regulation of the strength of synaptic outputs from rods and cones to second-order neurons.

Dopamine regulates the gap junction coupling between rods and cones. Activation of D2-type receptors enhances the rod–cone coupling in *Xenopus* retina at “mesopic” light intensities [43], at which both rods and cones contribute to visual responses. This coupling, which allows cone signals to flow into the rod pathway and vice versa, is likely to take advantage of both high sensitivity of rod inputs and high temporal resolution of the cone-driven circuitry. In bright light, however, D2-type receptors mediate the opposite effect. As demonstrated in the fish, mouse, and rabbit retinas, dopamine acting through D2-type receptors reduces rod–cone coupling in bright daylight, an effect also regulated by the circadian clock [40, 44, 45]. This uncoupling of rods from cones appears to be the best established mechanism of how dopamine prevents rod signals from flowing into the cone-driven circuitry in bright light.

The coupling state of horizontal cells also undergoes dopaminergic regulation. Zhang et al. showed that primate horizontal cells are electrically coupled in the dark and become uncoupled in the light, a mechanism mediated by activation of D1R [34]. Ribelayga and Mangel found that in bright light D1R activation reduces rod horizontal cell coupling in the fish retina [46]. Very similar observations were obtained by Weiler and Vaney for horizontal cells of the mouse retina [47]. This regulation may be further fine-tuned under dim light conditions when the degree of horizontal cell coupling is pronounced even more than in complete darkness [48].

Horizontal cells provide the antagonistic surround receptive fields for bipolar and ganglion cells [49–51], which is thought to contribute to enhanced contrast detection at the level of the outer plexiform layer [52]. Since horizontal cell uncoupling establishes smaller receptive field sizes [53], this mechanism may contribute to the higher spatial resolution characteristic for cone-driven vision, as compared to rod-dominant vision operating with large visual fields [11]. However, recent work argues against this view by providing evidence that horizontal cell coupling does not affect the spatial tuning at the level of ganglion cells [54, 55]. Therefore, the physiological significance of horizontal cell uncoupling in mediating cone-driven visual responses remains controversial.

AII amacrine cells are moderately coupled among themselves via gap junctions in the dark, and the extent of this coupling is further increased by dim-light illumination [56]. Dopamine, acting via D1R, closes the gap junctions among AII amacrine cells in bright light [30, 57], which reduces the extent of their coupling to the degree observed in dark-adapted retina [55]. The extensive coupling of AII amacrine cells in dim light has been suggested to allow the summation of light signals over a relatively large receptive field to enhance the synchronized transmission of rod signals above the background noise [58, 59]. The significance of such a mecha-

nism could be to increase the reliability of synaptic transmission throughout the rod-driven circuitry, although we find it puzzling that, just as in the case of horizontal cells, this mechanism is employed primarily in dim light but not in the darkness where it would appear to be equally beneficial. Therefore, future work is needed to fully understand the functional significance of light-dependent AII amacrine cell coupling/uncoupling. The reduction of AII amacrine cell coupling in bright light should contribute to the reduction of the size of visual fields, generally consistent with the concept of dopamine assisting the transition from rod- to cone-dominant vision.

As a part of the primary rod-driven circuitry, AII amacrine cells also form gap junctions with cone ON bipolar cells (Fig. 8.1), which constitute electrical synapses between these two cell types. In contrast to the AII–AII uncoupling, dopamine does not uncouple AII amacrine cells from cone ON bipolar cells [60], which would be an alternative mechanism to prevent light signals from rods to reach ganglion cells in bright light. On the contrary, there is evidence that dopamine exerts opposite effects in dim light ([61]; see the following section). Overall, it appears that dopamine acts to bias the retinal circuitry toward cone vision in bright light, but not to block rod signals entirely. Indeed, psychophysical studies report the presence of rod inputs under photopic conditions (e.g., [62]).

Ganglion cell coupling is also controlled by dopamine. The coupling between OFF α ganglion cells is maintained at a relatively low level in the dark due to the activity of D2-type dopamine receptors; the extent of this coupling increases in bright light, which is mediated by D1R [63]. Ganglion cell coupling is the cellular basis for synchronized firing between neighboring cells [64, 65]. This synchronized activity might serve to compress visual information into parallel streams for robust and reliable transmission via the optic nerve to visual centers in the brain [55]. Such a mechanism might ultimately facilitate the processing of visual signals by higher visual centers [66] and therefore become significant at brighter light levels when the retina integrates more complex light information than single photons. The full extent of the regulation of ganglion cell coupling and synchronized activity by dopamine and its relation to enhancement of cone vision is not fully explored and awaits further studies.

It should be added that the effects of dopamine on the coupling state of different retinal neurons poses an interesting problem regarding the underlying molecular mechanisms. Dopamine acting via D1R causes dephosphorylation of connexin Cx36 which forms the gap junctions in AII amacrine cells [67]. This dephosphorylation triggers gap junction closure and AII amacrine cell uncoupling by reducing the opening probability and opening frequency of the gap junction channels [68]. In this mechanism, D1R activates protein kinase A (PKA), which is thought to phosphorylate the PP2A phosphatase. Phosphorylated and thereby activated PP2A can now dephosphorylate Cx36 which results in the gap junction closure. On the other hand, dopamine acting via D2-type receptors in photoreceptors also causes dephosphorylation of Cx36 and, as a result, rod/cone uncoupling. Since D2-type receptors inhibit PKA activity (whereas D1R activates PKA), Cx36 has to be directly phosphorylated by PKA in this case to allow for D2R-mediated closing of gap junctions

[40]. Similarly, D1R stimulates ganglion cell coupling as described above via direct phosphorylation of Cx36 by PKA [55, 69]. How this differential regulation of Cx36 phosphorylation in individual types of retinal neurons is achieved on the molecular basis (e.g., whether PKA is engaged in signaling complexes that differ between photoreceptors, ganglion and AII amacrine cells) has not yet been resolved.

Several studies on lower vertebrates provide evidence that dopamine can reduce both light responses and light-dependent synaptic output from rods. Thoreson et al. showed that dopamine acting through D2-like receptors reduces the rod synaptic output in the amphibian retina [70]. Witkovsky et al. found that dopamine increases cone inputs to the axon-bearing horizontal cells of the *Xenopus* retina, while suppressing the rod input onto these cells [71]. Again, this effect was mediated by D2-like dopamine receptors. On the other hand, stimulation of D2-like receptors reduces the hyperpolarization-dependent I_h current in rods of *Xenopus* [72]. Since the I_h current counteracts the hyperpolarizing light responses in rods by depolarizing these cells, this could potentially lead to dopamine-dependent enhancement of rod responses to flash stimuli. However, the authors argued that this depolarization is important to reduce rod responses to temporally fluctuating bright light stimuli. The dopamine-dependent decrease of I_h would drive rods into saturation and, therefore, prevent them from transmitting light signals to second-order neurons.

In summary, the available experimental evidence leads to a picture in which dopamine reduces the rod-driven light signals at two major sites: at the electrical synapses between rods and cones formed by gap junctions, and at the synapses formed between rod terminals and downstream horizontal and bipolar cells. Dopamine's effect on the coupling between horizontal, amacrine and ganglion cells might serve to enhance the features of retinal processing which are characteristic for cone vision. Although dopamine suppresses the rod-driven circuitry in favor of the cone-driven pathway in bright light, the dopaminergic system has the remarkable capacity to operate in a reversed manner in dim light by suppressing cone-driven responses while facilitating rod-driven responses. The role of dopamine as a facilitator of rod vision will be described in the following sections.

Dopamine Regulates Retinal Circuitry in the Dark and in Dim Light

Despite dopamine's well-recognized role in adaptation to bright light, there is a large body of experimental evidence that dopamine also plays a critical role in regulating light responses of the retina adapted to the dark or dim light. One of the first publications demonstrating that dopamine regulates visual processing in the dark-adapted retina came from Mangel and Dowling performing single-cell recordings of cone horizontal cells in the goldfish [73]. The results obtained in that study indicated that in dark-adapted retina, dopamine release causes a reduction of both light responses and the receptive field size of cone horizontal cells. A similar result was reported by Yang et al. who found that dopamine blocks light responses of cone

horizontal cells in dark-adapted fish retina [33]. These data showed that dopamine not only suppresses the rod-mediated circuitry in favor of the cone-driven pathway in bright light but also acts in the opposite manner to silence cone inputs onto horizontal cells when vision is dominated by the rod-mediated circuitry in the dark or in dim light. Although these studies were performed in lower vertebrates, there is clear evidence for dopamine release in dark-adapted retinas of mammalian species. High steady-state level of dopamine release was observed in the rabbit retina [26], and significant dopamine synthesis and release were also documented in the mouse retina during the night [74].

Perhaps the first study that described a critical role of dopamine signaling in facilitating rod-driven responses was conducted with the zebra fish retina [61]. Depletion of dopaminergic amacrine cells caused an elevation of dark-adapted visual thresholds by as much as two to three orders of magnitude. This result was interpreted as a consequence of reduced dopamine levels, which impaired the rod-driven retinal output both at the level of ganglion cell activity and in behavioral assays. These findings showed that the presence of released dopamine in the dark-adapted retina is critical for rod signals to be effectively transmitted to ganglion cells.

More recent work conducted in the mouse provided evidence that at least one major target of dopamine in the dark and in dim light is the rod bipolar cell [20]. In this study, we and our colleagues demonstrated that dopamine is engaged in a mechanism that allows rod bipolar cells to maintain high light sensitivity over a wide range of light intensities. The experimental highlights from this study and detailed discussion of this mechanism are the subject of two subsequent sections.

The Role of Dopamine in Sensitizing and Light-Adapting the Rod-Driven Circuitry

The initial evidence that dopamine plays a critical role in sensitizing light responses of rod bipolar cells and that this function is conveyed specifically via D1R came from our electroretinography (ERG) analysis of knockout mice lacking each of the five dopamine receptors ($D1R^{-/-}$, $D2R^{-/-}$, $D3R^{-/-}$, $D4R^{-/-}$, and $D5R^{-/-}$ mice) [20]. ERG is a noninvasive technique consisting of recording massed field potentials generated by light responses of retinal neurons in vivo using an electrode placed at the cornea. ERG allows monitoring these responses without perturbing any neuronal connections, affecting endogenous neurotransmitter levels, or altering intra- and extracellular ion concentrations [75]. A typical dark-adapted ERG evoked by a dim flash consists predominantly of a positive signal, the “b-wave,” which reflects the cumulative depolarization of rod bipolar cells [75, 76].

Our experiments demonstrated that the amplitudes of ERG b-waves recorded from $D1R^{-/-}$ mice were smaller than those from wild-type (WT) controls, particularly in the presence of adapting background illumination (Fig. 8.3a). The analysis of rod-driven b-wave light sensitivities (defined as the ratio between the maximal b-wave amplitude and its half-saturating flash intensity) measured at various back-

ground light intensities showed that the lack of D1R reduces the rod bipolar cell operational range, i.e., the range of light intensities allowing reliable responses above the noise level (Fig. 8.3b). This reduction was not accompanied by any abnormality in the retinal morphology of *D1R*$^{-/-}$ mice, which suggested that it reflects a distinct functional, rather than anatomical, impairment of the rod-driven circuitry. In control experiments, the results obtained with *D1R*$^{-/-}$ mice were phenocopied by pharmacological blockade of D1R in WT mice using a D1R antagonist, SCH-23390. The reductions in b-wave response amplitude and sensitivity were specific for *D1R*$^{-/-}$ mice; these parameters were unaffected in mice lacking any of the four other dopamine receptors.

As described above, D1R is expressed rather ubiquitously throughout the entire retina. Although found in a subset of cone bipolar cells [32], D1R was not detected in rod bipolar cells [20]. This suggests that the D1R-dependent regulation of rod bipolar cells' light responses originates from dopamine modulating another neuron type(s). In this case, the immediate sensitization of rod bipolar cells is likely to be mediated by a different neurotransmitter. Indeed, our subsequent experiments argued that the effect of dopamine is conveyed via a GABAergic input. Earlier studies demonstrated that rod bipolar cells contain two types of chloride channel GABA receptors, $GABA_AR$ and $GABA_CR$ (e.g., [77–79]), and receive GABAergic inputs from amacrine and potentially horizontal cells [79–82]. Remarkably, ERG recordings from $GABA_CR$-knockout (*$GABA_CR^{-/-}$*) mice revealed a phenotype strikingly similar to that of *D1R*$^{-/-}$ mice, consisting of a substantial reduction in b-wave dark sensitivity and compression of b-wave operational range (Fig. 8.3c, d). The same effect was observed in WT mice following a pharmacological blockade of $GABA_CRs$ with intraocularly injected $GABA_CR$ antagonist, TPMPA. In contrast, the pharmacological blockade of $GABA_ARs$ did not affect either dark sensitivity or operational range of rod-driven b-waves.

These results suggested that the effect of D1R knockout can be explained by an alteration of the $GABA_CR$-mediated input onto rod bipolar cells. Further support for this idea came from a set of reciprocal experiments in which ERG responses were recorded after intraocular injections of GABA (Fig. 8.3e). GABA injections increased b-wave amplitudes in WT mice, but did not change b-wave sensitivity and operational range (Fig. 8.3f). On the contrary, intraocular GABA injections into *D1R*$^{-/-}$ mice not only increased b-wave amplitudes but also restored the b-wave light sensitivity and operational range to the levels observed in WT mouse controls. Therefore, the lack of D1R-mediated signaling could be completely compensated by exogenous GABA, further supporting the idea that dopamine binding to D1R induces a GABAergic input onto rod bipolar cells. Consistently, pharmacological blockade of $GABA_CR$ in *D1R*$^{-/-}$ mice did not further reduce b-wave sensitivity (Fig. 8.3d), indicating that the dopamine-dependent sensitization of rod bipolar cells is mediated by $GABA_CR$.

The next important question addressed the mechanism by which the GABAergic input mediated by $GABA_CRs$ sensitizes rod bipolar cells. Initial insights came from the analysis of maximal amplitudes of dark-adapted rod-driven ERG b-waves, which are proportional to the extent of bipolar cell depolarization upon a saturating

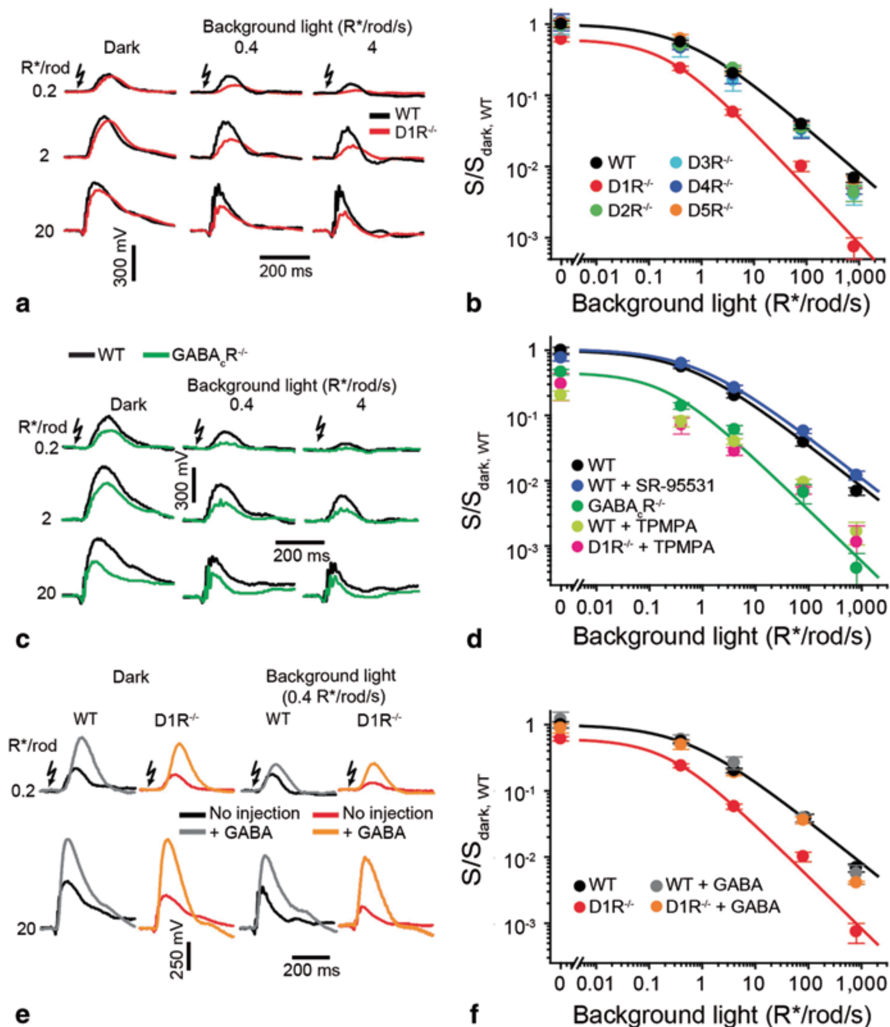

Fig. 8.3 Summary of the key ERG results providing evidence for the dopamine-dependent sensitization of rod bipolar cell light responses. **a** ERG recordings from wild-type (*WT*) and *D1R*$^{-/-}$ mice performed under dark- and light-adapted conditions. Representative ERG responses consisting predominantly of a positive deflection, the b-wave, which reflects the cumulative light activity of rod bipolar cells, are shown. Light intensities for flash and background light are provided in units of photoexcited rhodopsin molecules per rod (*R*/rod*) and photoexcited rhodopsin molecules per rod per second (*R*/rod/s*), respectively. **b** The sensitivities of rod-driven ERG b-waves (the ratio between the saturating b-wave amplitude and its half-saturating flash intensity) were determined for the five dopamine receptor knockout mice, normalized to the dark sensitivity of *WT* mice ($S/S_{dark,\ WT}$) and plotted as a function of background light intensity (mean ± SEM). **c** Representative ERG recordings from *WT* and $GABA_CR^{-/-}$ mice under dark- and light-adapted conditions. **d** ERG b-wave sensitivity plots for the following mice and conditions: *WT*; $GABA_CR^{-/-}$; *WT* intraocularly injected with the $GABA_A$R antagonist, SR-95531; *WT* intraocularly injected with the $GABA_C$R antagonist, *TPMPA*; *D1R*$^{-/-}$ intraocularly injected with TPMPA. **e** ERG recordings from dark- or light-adapted *WT* and D1R$^{-/-}$ mice with and without intraocular *GABA* injections. **f** ERG b-wave sensitivity plots for *WT* and *D1R*$^{-/-}$ mice with or without intraocular injections of *GABA* (mean ± SEM).

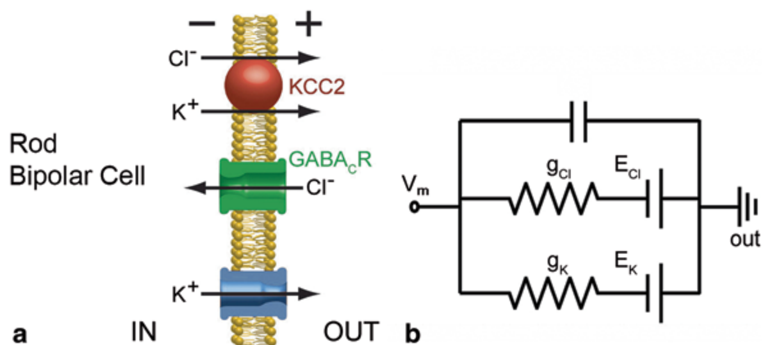

Fig. 8.4 Both potassium and sustained chloride currents contribute to hyperpolarization of the rod bipolar cell resting membrane potential. **a** Cartoon illustrating the role of KCC2 in chloride extrusion from rod bipolar cells and the roles of sustained chloride and potassium currents in hyperpolarization of the rod bipolar cell resting potential. **b** Electrical equivalent circuit illustrating the exchangeability and additivity of chloride and potassium conductances in hyperpolarization of the rod bipolar cell resting potential and in creating the driving force for light-induced cation influx causing cell depolarization. Based on this equivalent circuit, the resting potential is defined as $V_m = (g_K \cdot E_K + g_{Cl} \cdot E_{Cl})/(g_K + g_{Cl})$, where g_K and g_{Cl} are potassium and chloride conductances, and E_K and E_{Cl} are potassium and chloride reversal potentials, respectively

light stimulus [76]. This saturating response amplitude was found to be significantly reduced in both $D1R^{-/-}$ and $GABA_CR^{-/-}$ mice, which suggested that sustained chloride currents carried by $GABA_C$Rs extend the voltage range between resting potential and maximal light-evoked depolarization. On the other hand, dark-adapted b-wave amplitude was ~twofold increased upon intraocular injections of GABA, which indicated that this voltage range could be significantly expanded beyond the normal WT level when additional $GABA_C$R channels are allowed to be opened.

A sustained $GABA_C$R-mediated chloride current could extend the voltage range of rod bipolar cell responses by contributing to hyperpolarization of these cells' resting potential. In agreement with this notion, patch clamp recordings from rod bipolar cells showed that their light response amplitudes increase with more negative values of the holding potential [83].

A prerequisite for this chloride-dependent hyperpolarization is that the chloride equilibrium potential in the resting state is negative to the rod bipolar cells' resting potential. Only in this case, the chloride influx would hyperpolarize the cell in the same manner in which potassium outflux fulfills this function in other neurons (e.g., [84]). Should this condition be met, the electrochemical gradients of both chloride and potassium would cause hyperpolarization of the rod bipolar cell resting potential and provide the electrical driving force for the light-induced cation influx. This concept and an equivalent circuit describing the respective contributions from chloride and potassium currents to rod bipolar cell hyperpolarization are illustrated in Fig. 8.4.

The large transmembrane gradient of chloride in rod bipolar cells is maintained primarily by the K^+/Cl^- co-transporter KCC2, which extrudes chloride from these

cells utilizing the chemical gradient of potassium as the driving force. Immunolabeling indicates that KCC2 is expressed throughout most parts of the rod bipolar cell [85, 86], being most abundant in axons and cell bodies [20, 85, 86]. We should add that an early study suggested that ON bipolar cells also express the NKCC1 transporter localized to their dendrites. Because NKCC1 accumulates chloride inside the cells, the authors proposed that chloride is transported in opposite directions in axons and dendrites of rod bipolar cells [87]. However, they revised this conclusion in a subsequent study after gaining access to the NKCC1-knockout mouse, which allowed them to evaluate the immunostaining specificity of their antibodies [86]. They found that NKCC1 immunostaining in rod bipolar cell dendrites was due to cross-reactivity of the "T4" anti-NKCC1 antibody used in their original experiments. They used another, non-cross-reacting antibody to demonstrate that, within the outer plexiform layer, NKCC1 is expressed only in horizontal cells but not in bipolar cell dendrites [86]. Regrettably, this critical revision is not always appreciated by the community (e.g., [88]). Another argument against the possibility that chloride is transported in opposite directions at dendrites and axon terminals of rod bipolar cells was obtained in single-cell recordings by Satoh et al. who showed that the values for chloride reversal potentials are the same at the dendritic and axonal ends of these cells [89]. This result is consistent with rod bipolar cells expressing KCC2 but not NKCC1.

The functional evidence that KCC2 plays an important role in creating a chloride gradient across the rod bipolar cell plasma membrane in vivo was obtained by pharmacological blockade of KCC2 in WT mice. This blockade reduced the dark-sensitivity and operational range of ERG b-waves very similarly to $GABA_CR$ inactivation [20]. Importantly, this effect of KCC2 blockade could not be restored by exogenous GABA; $GABA_CR$ opening in this case could not hyperpolarize the cell due to a disrupted chloride gradient caused by the KCC2 inactivation. In addition to KCC2, rod bipolar cells express the sodium-driven chloride bicarbonate exchanger NCBE [88], which also extrudes chloride from these cells [90]. Therefore, it is likely that the combined action of both KCC2 and NCBE establishes the physiological chloride gradient across the plasma membrane of rod bipolar cells. This idea is consistent with the observation that NCBE-knockout mice display reduced ERG b-wave amplitudes in the dark [88], again most likely due to a disrupted chloride gradient.

Another requirement for this chloride-dependent hyperpolarization mechanism is that the GABA receptor chloride channels do not undergo ligand-dependent inactivation over time. In this context, $GABA_CRs$ represent a perfect fit due to lack of their GABA-dependent desensitization [91]. This property distinguishes $GABA_CRs$ from γ subunit-containing $GABA_AR$ channels which are localized to postsynaptic sites of the synaptic cleft. These γ subunit-containing $GABA_ARs$ are activated by transient synaptic GABA release and mediate transient inhibition. Furthermore, these receptors undergo rapid GABA-dependent desensitization (e.g., [92, 93]) and therefore are better suited to provide dynamic GABAergic inhibitory feedbacks than long-lasting modulation. In contrast, the δ subunit-containing $GABA_ARs$ are localized to extrasynaptic sites of multiple neurons where they mediate tonic inhibition,

similar to the role of $GABA_C$Rs in rod bipolar cells. For example, $GABA_A$Rs consisting of $\alpha_6\beta\delta$ subunits in cerebellar granule cells and of $\alpha_4\beta\delta$ subunits in hippocampal dentate granule cells have a very high affinity for GABA and can be activated by the low ambient concentration of GABA present in the extracellular space [94]. Consistent with their role, the δ subunit-containing $GABA_A$Rs are also resistant to desensitization, similar to $GABA_C$Rs [95].

Further experiments in WT mouse retinal slices confirmed the presence of a tonic GABAergic current in rod bipolar cells that was antagonized by the $GABA_C$R blocker, TPMPA [20]. Consistently, this current was absent in rod bipolar cells of $GABA_C$R-knockout retinas. As expected, this sustained $GABA_C$R-mediated current hyperpolarized the rod bipolar cell resting potential. The latter conclusion came from experiments in which we measured the resting membrane potential in current clamp with zero holding current. TPMPA depolarized the resting potential of WT rod bipolar cells, but had no effect on rod bipolar cells in $GABA_C$R-knockout mice.

In summary, dopamine acting via D1R induces a sustained release of GABA from one or several types of retinal neurons, which supports a $GABA_C$R-carried chloride current hyperpolarizing the resting potential of rod bipolar cells. This hyperpolarization increases the amplitude of rod bipolar cell light responses, both in the dark and in the presence of background illumination thereby extending the operational range of these cells. The physiological significance of such a nonconventional hyperpolarization mechanism may be rationalized as follows. Rod bipolar cells have to maintain a large chloride gradient in order to receive the strong dynamic GABAergic feedback that shapes their light responses. However, the generation of this chloride gradient, which is accomplished by KCC2 co-transporting both K^+ and Cl^- outside the cell, comes at the expense of reducing the gradient of potassium. This creates a potential problem whereby the diminished driving force for potassium results in hyperpolarization insufficient to maintain high light sensitivity of these cells. This problem is solved by using the newly acquired driving force for chloride to compensate for the “missing” component of rod bipolar cell hyperpolarization.

Potential Cellular Sites for Sustained Dopamine-Dependent GABA Release

A central question emerging from our study [20] is the identity of retinal neuron(s) providing the sustained D1R-dependent GABAergic input onto rod bipolar cells. As described above, D1R is expressed in horizontal and amacrine cells, both forming synaptic connections with rod bipolar cells in the outer and inner plexiform layers, respectively (Fig. 8.1). Additionally, $GABA_C$Rs are localized to both axon terminals and dendrites of rod bipolar cells [20, 79], and the expression pattern of KCC2 suggests that efficient chloride extrusion could take place over the entire rod bipolar cell. Therefore, both amacrine and horizontal cells could potentially serve as the source of sustained GABAergic inputs onto rod bipolar cells.

Little additional information is currently available to further differentiate between amacrine cells and horizontal cells (or both) responsible for sustained GABA release. The only experimental hint so far came from the analysis of light-dependent GABA immunostaining of amacrine and horizontal cells in WT and *D1R*$^{-/-}$ mice [20]. Whereas GABA staining in amacrine cells did not reveal any systematic light-dependent changes in either animal type, the GABA staining of horizontal cells in WT mice increased upon illumination. This light dependency was abolished in *D1R*$^{-/-}$ mice, in which the GABA staining remained at a constant high level regardless of conditions of illumination. One way to interpret this result is to suggest that GABA staining intensity correlates with the intracellular retention of GABA, which could serve as a reciprocal measure of the amount of GABA released. Based on this interpretation, horizontal cells would release less GABA in *D1R*$^{-/-}$ than in WT mice and produce reduced GABAergic inputs onto rod bipolar cells.

On the other hand, the hypothesis that horizontal cells serve as a major site for sustained GABA release faces a major problem. The largest phenotypes of both D1R and $GABA_C$R knockouts on ERG b-wave sensitivity are observed in the presence of background light which causes horizontal cells to hyperpolarize (e.g., [96]). In contrast, GABA release from any neuron requires its depolarization, no matter whether release occurs via synaptic or inversed transport mechanism [97, 98]. Indeed, depolarization induces GABA release from isolated horizontal cell preparations [99]. Whereas one can speculate that dopamine counteracts this light-dependent hyperpolarization [71, 100], the net effect of light exposure results in horizontal cell hyperpolarization. This leaves a possibility for horizontal cells to provide sustained GABA release in the dark, but casts doubt that they can do it in light-adapted retinas.

Given these considerations, sustained GABA release could originate from amacrine cells. Specifically, GABAergic inhibition from amacrine cells can be seen at either the reciprocal synapses formed between rod bipolar cell axon terminals and of the A17 amacrine cell or at more typical inhibitory synapses formed from wide-field amacrine cells onto rod bipolar cells [77, 101, 102]. Consistent with this hypothesis, sustained $GABA_C$R-mediated currents have been documented in axon terminals of mixed rod/cone bipolar cells of the goldfish retina [103, 104]. Furthermore, experiments in retinal slices demonstrated that both $GABA_C$R and $GABA_A$R antagonists decrease the dynamic range of light responses in intact rod bipolar cells, but not in cells with severed axon terminals [83]. Because the available evidence for the cellular origin of sustained GABA release does not allow a distinct answer, there is a need for additional experiments to elucidate the contributions from horizontal and amacrine cells to this regulatory mechanism.

Conclusions and Future Directions

The data reviewed in this chapter summarize evidence for a novel role of dopamine in the retinal circuitry. They expand the well-established role of dopamine as a messenger of bright-light adaptation to a sensitizer of the rod-driven circuitry for

dim-light vision. Dopamine exerts this new function via binding to D1R receptors in a yet-to-be identified retinal neuron(s), which induces a sustained hyperpolarizing GABAergic input onto rod bipolar cells. The $GABA_C$R-dependent chloride current produced by this input is critical for hyperpolarization of the rod bipolar cell's resting potential. As a result, the rod bipolar cell utilizes an atypical strategy for hyperpolarizing its resting potential by employing not only potassium currents, as most neurons do, but also chloride currents. Electrochemically, the contributions of these two currents are interchangeable and additive. However, the potential regulation of these currents is different. While the potassium conductance is defined by the intrinsic properties of channels and transporters expressed in rod bipolar cells, the chloride conductance is modulated by sustained GABAergic inputs from a GABA-releasing cell, a process further regulated by the dopamine content in the retina.

Once the cellular origin of sustained GABA release becomes established, the next question to address would be whether the sustained GABAergic input could be dynamically regulated by the changes in ambient illumination and whether such a regulation is based on the well-documented light-dependent increase in retinal dopamine levels. Another experimental direction would be to elucidate the intracellular molecular mechanism responsible for supporting the D1R-dependent GABA release.

It is an open question whether a similar dopamine-dependent sensitizing and range-extending mechanism applies to any of the cone bipolar cells as well. Interestingly, recent work on the zebra fish retina showed that the *celsr3* mutation causes an increase in both $GABA_C$R and $GABA_A$R expression at the axon terminals of ON bipolar cells [105], which receive either cone or mixed rod–cone inputs [106]. This overexpression resulted in increased b-wave amplitudes, similar to the effect of exogenous GABA on rod-driven b-wave responses in the mouse [20, 76]. This observation suggests that sustained chloride currents carried by GABA receptors could indeed play a general role in sensitizing light responses of both rod- and cone-driven ON bipolar cells, adding yet another function for dopamine in the retina.

Acknowledgments This work was supported by the NIH Grants C06-RR016511 (MCW Eye Institute), P30-EY001931 (MCW), EY10336 (V.Y.A.), and EY5722 (Duke University), and unrestricted departmental grants from Research to Prevent Blindness to MCW Eye Institute and Duke University.

References

1. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors: from structure to function. Physiol Rev 78(1):189–225
2. Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63(1):182–217
3. Diehl DJ, Gershon S (1992) The role of dopamine in mood disorders. Compr Psychiatry 33(2):115–120
4. Kulisevsky J (2000) Role of dopamine in learning and memory: implications for the treatment of cognitive dysfunction in patients with Parkinson's disease. Drugs Aging 16(5):365–379

5. Snyder SH (1976) The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. Am J Psychiatry 133(2):197–202
6. Lorea I, Fernandez-Montalvo J, Tirapu-Ustarroz J, Landa N, Lopez-Goni JJ (2010) Neuropsychological performance in cocaine addiction: a critical review. Rev Neurol 51(7):412–426
7. Hasbi A, Fan T, Alijaniaram M, Nguyen T, Perreault ML, O'Dowd BF et al (2009) Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. Proc Natl Acad Sci U S A 106(50):21377–21382
8. Piomelli D, Pilon C, Giros B, Sokoloff P, Martres MP, Schwartz JC (1991) Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism. Nature 353(6340):164–167
9. Beaulieu JM, Gainetdinov RR, Caron MG (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends PharmacolSci 28(4):166–172
10. Beaulieu JM, Del'guidice T, Sotnikova TD, Lemasson M, Gainetdinov RR (2011) Beyond cAMP: the regulation of Akt and GSK3 by dopamine receptors. Front Mol Neurosci 4:38
11. Witkovsky P (2004) Dopamine and retinal function. Doc Ophthalmol 108(1):17–40
12. Rodieck RW (1998) The first steps in seeing. Sinauer Associates, Sunderland
13. Dunn FA, Doan T, Sampath AP, Rieke F (2006) Controlling the gain of rod-mediated signals in the Mammalian retina. J Neurosci 26(15):3959–3970
14. Shapley RM, Enroth-Cugell C (1984) Visual adaptation and retinal gain controls. Prog Retin Res 3:263–346
15. Dowling JE, Ehinger B (1975) Synaptic organization of the amine-containing interplexiform cells of the goldfish and Cebus monkey retinas. Science 188(4185):270–273
16. Dacey DM (1990) The dopaminergic amacrine cell. J Comp Neurol 301(3):461–489
17. Kolb H, Cuenca N, Wang HH, Dekorver L (1990) The synaptic organization of the dopaminergic amacrine cell in the cat retina. J Neurocytol 19(3):343–366
18. Gustincich S, Feigenspan A, Wu DK, Koopman LJ, Raviola E (1997) Control of dopamine release in the retina: a transgenic approach to neural networks. Neuron 18(5):723–736
19. Savy C, Moussafi F, Durand J, Yelnik J, Simon A, Nguyen-Legros J (1995) Distribution and spatial geometry of dopamine interplexiform cells in the retina. II. External arborizations in the adult rat and monkey. J Comp Neurol 355(3):392–404
20. Herrmann R, Heflin SJ, Hammond T, Lee B, Wang J, Gainetdinov RR et al (2011) Rod vision is controlled by dopamine-dependent sensitization of rod bipolar cells by GABA. Neuron 72(1):101–110
21. Hoshi H, Liu WL, Massey SC, Mills SL (2009) ON inputs to the OFF layer: bipolar cells that break the stratification rules of the retina. J Neurosci 29(28):8875–8883
22. Zhang DQ, Zhou TR, McMahon DG (2007) Functional heterogeneity of retinal dopaminergic neurons underlying their multiple roles in vision. J Neurosci 27(3):692–699
23. Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG (2008) Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. Proc Natl Acad Sci U S A 105(37):14181–14186
24. Newkirk GS, Hoon M, Wong RO, Detwiler PB (2013) Inhibitory inputs tune the light response properties of dopaminergic amacrine cells in mouse retina. J Neurophysiol 110:536–552
25. Puopolo M, Hochstetler SE, Gustincich S, Wightman RM, Raviola E (2001) Extrasynaptic release of dopamine in a retinal neuron: activity dependence and transmitter modulation. Neuron 30(1):211–225
26. Mills SL, Xia XB, Hoshi H, Firth SI, Rice ME, Frishman LJ et al (2007) Dopaminergic modulation of tracer coupling in a ganglion-amacrine cell network. Vis Neurosci 24(4):593–608
27. Weiler R, Baldridge WH, Mangel SC, Dowling JE (1997) Modulation of endogenous dopamine release in the fish retina by light and prolonged darkness. Vis Neurosci 14(2):351–356
28. Bjelke B, Goldstein M, Tinner B, Andersson C, Sesack SR, Steinbusch HW et al (1996) Dopaminergic transmission in the rat retina: evidence for volume transmission. J Chem Neuroanat 12(1):37–50
29. Pourcho RG (1982) Dopaminergic amacrine cells in the cat retina. Brain Res 252(1):101–109

30. Hampson EC, Vaney DI, Weiler R (1992) Dopaminergic modulation of gap junction permeability between amacrine cells in mammalian retina. J Neurosci 12(12):4911–4922
31. Versaux-Botteri C, Gibert JM, Nguyen-Legros J, Vernier P (1997) Molecular identification of a dopamine D1b receptor in bovine retinal pigment epithelium. Neurosci Lett 237(1):9–12
32. Veruki ML, Wassle H (1996) Immunohistochemical localization of dopamine D1 receptors in rat retina. Eur J Neurosci 8(11):2286–2297
33. Yang XL, Tornqvist K, Dowling JE (1988) Modulation of cone horizontal cell activity in the teleost fish retina. II. Role of interplexiform cells and dopamine in regulating light responsiveness. J Neurosci 8(7):2269–2278
34. Zhang AJ, Jacoby R, Wu SM (2011) Light- and dopamine-regulated receptive field plasticity in primate horizontal cells. J Comp Neurol 519(11):2125–2134
35. Hayashida Y, Rodriguez CV, Ogata G, Partida GJ, Oi H, Stradleigh TW et al (2009) Inhibition of adult rat retinal ganglion cells by D1-type dopamine receptor activation. J Neurosci 29(47):15001–15016
36. Cohen AI, Todd RD, Harmon S, O'Malley KL (1992) Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. Proc Natl Acad Sci U S A 89(24):12093–12097
37. Muresan Z, Besharse JC (1993) D2-like dopamine receptors in amphibian retina: localization with fluorescent ligands. J Comp Neurol 331(2):149–160
38. Pozdeyev N, Tosini G, Li L, Ali F, Rozov S, Lee RH et al (2008) Dopamine modulates diurnal and circadian rhythms of protein phosphorylation in photoreceptor cells of mouse retina. Eur J Neurosci 27(10):2691–2700
39. Veruki ML (1997) Dopaminergic neurons in the rat retina express dopamine D2/3 receptors. Eur J Neurosci 9(5):1096–1100
40. Li H, Zhang Z, Blackburn MR, Wang SW, Ribelayga CP, O'Brien J (2013) Adenosine and dopamine receptors coregulate photoreceptor coupling via gap junction phosphorylation in mouse retina. J Neurosci 33(7):3135–3150
41. Jackson CR, Ruan GX, Aseem F, Abey J, Gamble K, Stanwood G et al (2012) Retinal dopamine mediates multiple dimensions of light-adapted vision. J Neurosci 32(27):9359–9368
42. Mangel SC, Ribelayga C (2011) The Circadian clock in the retina regulates rod and cone pathways. In: Besharse JC, Bock D (eds) The retina and its disorders, Academic, Oxford, pp 105–111
43. Krizaj D (2000) Mesopic state: cellular mechanisms involved in pre- and post-synaptic mixing of rod and cone signals. Microsc Res Tech 50(5):347–359
44. Ribelayga C, Cao Y, Mangel SC (2008) The circadian clock in the retina controls rod-cone coupling. Neuron 59(5):790–801
45. Ribelayga C, Mangel SC (2010) Identification of a circadian clock-controlled neural pathway in the rabbit retina. PLoS ONE 5(6):e11020
46. Ribelayga C, Mangel SC (2007) Tracer coupling between fish rod horizontal cells: modulation by light and dopamine but not the retinal circadian clock. Vis Neurosci 24(3):333–344
47. He S, Weiler R, Vaney DI (2000) Endogenous dopaminergic regulation of horizontal cell coupling in the mammalian retina. J Comp Neurol 418(1):33–40
48. Xin D, Bloomfield SA (1999) Dark- and light-induced changes in coupling between horizontal cells in mammalian retina. J Comp Neurol 405(1):75–87
49. Mangel SC, Miller RF (1987) Horizontal cells contribute to the receptive field surround of ganglion cells in the rabbit retina. Brain Res 414(1):182–186
50. Marchiafava PL (1978) Horizontal cells influence membrane potential of bipolar cells in the retina of the turtle. Nature 275(5676):141–142
51. Naka KI, Nye PW (1971) Role of horizontal cells in organization of the catfish retinal receptive field. J Neurophysio l34(5):785–801
52. Masland RH (2001) The fundamental plan of the retina. Nat Neurosci 4(9):877–886
53. Shelley J, Dedek K, Schubert T, Feigenspan A, Schultz K, Hombach S et al (2006) Horizontal cell receptive fields are reduced in connexin57-deficient mice. Eur J Neurosci 23(12):3176–3186

54. Dedek K, Pandarinath C, Alam NM, Wellershaus K, Schubert T, Willecke K et al (2008) Ganglion cell adaptability: does the coupling of horizontal cells play a role? PLoS ONE 3(3):e1714
55. Bloomfield SA, Volgyi B (2009) The diverse functional roles and regulation of neuronal gap junctions in the retina. Nat Rev Neurosci 10(7):495–506
56. Bloomfield SA, Xin D, Osborne T (1997) Light-induced modulation of coupling between aII amacrine cells in the rabbit retina. Vis Neurosci 14(3):565–576
57. Kothmann WW, Massey SC, O'Brien J (2009) Dopamine-stimulated dephosphorylation of connexin 36 mediates aII amacrine cell uncoupling. J Neurosci 29(47):14903–14911
58. Smith RG, Vardi N (1995) Simulation of the aII amacrine cell of mammalian retina: functional consequences of electrical coupling and regenerative membrane properties. Vis Neurosci 12(5):851–860
59. Vardi N, Smith RG (1996) The AII amacrine network: coupling can increase correlated activity. Vision Res 36(23):3743–3757
60. Mills SL, Massey SC (1995) Differential properties of two gap junctional pathways made by aII amacrine cells. Nature 377(6551):734–737
61. Li L, Dowling JE (2000) Effects of dopamine depletion on visual sensitivity of zebrafish. J Neurosci 20(5):1893–1903
62. Makous W (2001) Scotopic vision. In: Chalupa LM, Werner JH (eds) Visual neurosciences, MIT Press, Cambridge, pp 215–233
63. Hu EH, Pan F, Volgyi B, Bloomfield SA (2010) Light increases the gap junctional coupling of retinal ganglion cells. J Physiol 588(Part 21):4145–4163
64. Brivanlou IH, Warland DK, Meister M (1998) Mechanisms of concerted firing among retinal ganglion cells. Neuron 20(3):527–539
65. Mastronarde DN (1983) Interactions between ganglion cells in cat retina. J Neurophysiol 49(2):350–365
66. Puchalla JL, Schneidman E, Harris RA, Berry MJ (2005) Redundancy in the population code of the retina. Neuron 46(3):493–504
67. Feigenspan A, Teubner B, Willecke K, Weiler R (2001) Expression of neuronal connexin36 in AII amacrine cells of the mammalian retina. J Neurosci 21(1):230–239
68. McMahon DG, Knapp AG, Dowling JE (1989) Horizontal cell gap junctions: single-channel conductance and modulation by dopamine. Proc Natl Acad Sci U S A 86(19):7639–7643
69. Hidaka S, Kato T, Miyachi E (2002) Expression of gap junction connexin36 in adult rat retinal ganglion cells. J Integr Neurosci 1(1):3–22
70. Thoreson WB, Stella SL Jr, Bryson EI, Clements J, Witkovsky P (2002) D2-like dopamine receptors promote interactions between calcium and chloride channels that diminish rod synaptic transfer in the salamander retina. Vis Neurosci 19(3):235–247
71. Witkovsky P, Stone S, Besharse JC (1988) Dopamine modifies the balance of rod and cone inputs to horizontal cells of the Xenopus retina. Brain Res 449(1–2):332–336
72. Akopian A, Witkovsky P (1996) D2 dopamine receptor-mediated inhibition of a hyperpolarization-activated current in rod photoreceptors. J Neurophysiol 76(3):1828–1835
73. Mangel SC, Dowling JE (1985) Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. Science 229(4718):1107–1109
74. Nir I, Haque R, Iuvone PM (2000) Diurnal metabolism of dopamine in the mouse retina. Brain Res 870(1–2):118–125
75. Robson JG, Frishman LJ (1998) Dissecting the dark-adapted electroretinogram. Doc Ophthalmol 95(3–4):187–215
76. Robson JG, Maeda H, Saszik SM, Frishman LJ (2004) In vivo studies of signaling in rod pathways of the mouse using the electroretinogram. Vision Res 44(28):3253–3268
77. Chavez AE, Grimes WN, Diamond JS (2010) Mechanisms underlying lateral GABAergic feedback onto rod bipolar cells in rat retina. J Neurosci 30(6):2330–2339
78. Lukasiewicz PD, Shields CR (1998) A diversity of GABA receptors in the retina. Semin Cell Dev Biol 9(3):293–299

79. McCall MA, Lukasiewicz PD, Gregg RG, Peachey NS (2002) Elimination of the rho1 subunit abolishes GABA(C) receptor expression and alters visual processing in the mouse retina. J Neurosci 22(10):4163–4174
80. Suzuki S, Tachibana M, Kaneko A (1990) Effects of glycine and GABA on isolated bipolar cells of the mouse retina. J Physiol 421:645–662
81. Tachibana M, Kaneko A (1987) Gamma-Aminobutyric acid exerts a local inhibitory action on the axon terminal of bipolar cells: evidence for negative feedback from amacrine cells. Proc Natl Acad Sci U S A 84(10):3501–3505
82. Yang XL, Wu SM (1991) Feedforward lateral inhibition in retinal bipolar cells: input-output relation of the horizontal cell-depolarizing bipolar cell synapse. Proc Natl Acad Sci U S A 88(8):3310–3313
83. Euler T, Masland RH (2000) Light-evoked responses of bipolar cells in a mammalian retina. J Neurophysiol 83(4):1817–1829
84. Tessier-Lavigne M, Attwell D, Mobbs P, Wilson M (1988) Membrane currents in retinal bipolar cells of the axolotl. J Gen Physiol 91(1):49–72
85. Vu TQ, Payne JA, Copenhagen DR (2000) Localization and developmental expression patterns of the neuronal K-Cl cotransporter (KCC2) in the rat retina. J Neurosci 20(4):1414–1423
86. Zhang LL, Delpire E, Vardi N (2007) NKCC1 does not accumulate chloride in developing retinal neurons. J Neurophysiol 98(1):266–277
87. Vardi N, Zhang LL, Payne JA, Sterling P (2000) Evidence that different cation chloride cotransporters in retinal neurons allow opposite responses to GABA. J Neurosci 20(20):7657–7663
88. Hilgen G, Huebner AK, Tanimoto N, Sothilingam V, Seide C, Garrido MG et al (2012) Lack of the sodium-driven chloride bicarbonate exchanger NCBE impairs visual function in the mouse retina. PLoS ONE 7(10):e46155
89. Satoh H, Kaneda M, Kaneko A (2001) Intracellular chloride concentration is higher in rod bipolar cells than in cone bipolar cells of the mouse retina. Neurosci Lett 310(2–3):161–164
90. Wang CZ, Yano H, Nagashima K, Seino S (2000) The Na^+-driven Cl^-/HCO_3^- exchanger. Cloning, tissue distribution, and functional characterization. J Biol Chem 275(45):35486–35490
91. Amin J, Weiss DS (1994) Homomeric rho 1 GABA channels: activation properties and domains. Receptors Channels 2(3):227–236
92. Overstreet LS, Jones MV, Westbrook GL (2000) Slow desensitization regulates the availability of synaptic GABA(A) receptors. J Neurosci 20(21):7914–7921
93. Tia S, Wang JF, Kotchabhakdi N, Vicini S (1996) Distinct deactivation and desensitization kinetics of recombinant GABAA receptors. Neuropharmacology 35(9–10):1375–1382
94. Brickley SG, Mody I (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron 73(1):23–34
95. Mody I, Pearce RA (2004) Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci 27(9):569–575
96. Trumpler J, Dedek K, Schubert T, de Sevilla MLP, Seeliger M, Humphries P et al (2008) Rod and cone contributions to horizontal cell light responses in the mouse retina. J Neurosci 28(27):6818–6825
97. Koch U, Magnusson AK (2009) Unconventional GABA release: mechanisms and function. Curr Opin Neurobiol 19(3):305–310
98. Roth FC, Draguhn A (2012) GABA metabolism and transport: effects on synaptic efficacy. Neural Plast 2012:805830
99. Schwartz EA (1987) Depolarization without calcium can release gamma-aminobutyric acid from a retinal neuron. Science 238(4825):350–355
100. Hankins M, Ikeda H (1994) Early abnormalities of retinal dopamine pathways in rats with hereditary retinal dystrophy. Doc Ophthalmol 86(3):325–334
101. Chavez AE, Singer JH, Diamond JS (2006) Fast neurotransmitter release triggered by Ca influx through AMPA-type glutamate receptors. Nature 443(7112):705–708

102. Eggers ED, Lukasiewicz PD (2006) GABA(A), GABA(C) and glycine receptor-mediated inhibition differentially affects light-evoked signalling from mouse retinal rod bipolar cells. J Physiol 572(Pt 1):215–225
103. Hull C, Li GL, von Gersdorff H (2006) GABA transporters regulate a standing GABAC receptor-mediated current at a retinal presynaptic terminal. J Neurosci 26(26):6979–6984
104. Jones SM, Palmer MJ (2009) Activation of the tonic GABAC receptor current in retinal bipolar cell terminals by nonvesicular GABA release. J Neurophysiol 102(2):691–699
105. Lewis A, Wilson N, Stearns G, Johnson N, Nelson R, Brockerhoff SE (2011) Celsr3 is required for normal development of GABA circuits in the inner retina. PLoS Genet 7(8):e1002239
106. Li YN, Tsujimura T, Kawamura S, Dowling JE (2012) Bipolar cell-photoreceptor connectivity in the zebrafish (Danio rerio) retina. J Comp Neurol 520(16):3786–3802

Chapter 9
Regulation of Electrical Synaptic Plasticity in the Retina by G-Protein-Coupled Receptors

John O'Brien

Abstract More than six decades of research has firmly established the critical role of electrical synapses in retinal circuitry and physiology. The functional correlate of gap junctions between neural elements, electrical synapses serve unique purposes separate from those of chemical synapses. Electrical synapses between like neurons expand receptive field sizes, dampen noncorrelated noise, and coordinate activity. Between dissimilar cell types, electrical synapses establish specialized feed-forward synaptic circuits that play a prominent role in retinal signaling. Many electrical synapses display a high degree of plasticity, exceeding an order of magnitude in dynamic range that is an important component of circuit remodeling during light adaptation and circadian tuning of retinal function. This plasticity is largely dependent on activities of G-protein-coupled receptors responding to extracellular cues. The molecular mechanisms that transduce these signals are varied and specific to each cell type. Dopamine D1, and in some cases D2-like receptors, control coupling in horizontal cell, amacrine cell, and ganglion cell networks. In the AII amacrine cell, a balance between activity-dependent signaling that enhances coupling and dopamine-driven signaling that reduces coupling sets the functional state of each electrical synapse independently. An intricate balance of dopamine D4 and adenosine A2a and A1 receptor activities tightly controls photoreceptor coupling in a push–pull manner. This chapter provides a detailed view of the dynamic changes of electrical synapses in retinal neurons and the molecular mechanisms that control them.

J. O'Brien (✉)
The Richard S. Ruiz, M.D. Department of Ophthalmology and Visual Science
and The Graduate School of Biomedical Sciences, The University of Texas Houston
Medical School, 6431 Fannin St., MSB 7.024, Houston, TX 77030, USA
e-mail: John.OBrien@uth.tmc.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_9

Introduction

Electrical synapses are a unique form of synaptic communication that permits direct transfer of electric current between two cells. Formed by head-to-head docking of gap junction proteins from the two contacting cells, electrical synapses contain clusters of channels that can remain persistently open, allowing passage of electrical current and some cytoplasmic small molecules. Electrical synapses serve unique purposes separate from those of chemical synapses. Electrical synapses between like neurons expand receptive field sizes, dampen noncorrelated noise, and coordinate activity. Between dissimilar cell types, they establish specialized feed-forward synaptic circuits that play a prominent role in retinal signaling.

Many electrical synapses display a high degree of plasticity, exceeding an order of magnitude in dynamic range. This plasticity plays an important role in remodeling retinal circuits to tune the retina to function optimally in very different light regimes. The changes in electrical coupling can be driven by circadian rhythms or by light adaptation, and depend on a variety of mechanisms. This chapter provides a historical introduction to the discovery and physiological understanding of electrical synapses in the retina and examines in depth their plasticity, particularly with regard to its control by G-protein-coupled receptors. Several recent reviews provide more detailed views of the physiological roles and properties of electrical synapses in the retina and throughout the central nervous system [1, 2], and of their pathophysiologic roles in neuronal injury [3].

Historical Overview of Electrical Synapses in the Retina

Electrical synapses are the functional correlate of gap junctions between neural elements. First described in the giant septate motor axons of crayfish [4, 5], electrical synapses were shortly found to be widely distributed throughout the nervous system of vertebrates [6, 7]. Ultrastructural evidence of electrical synapses in retinal neurons was developing concomitantly with the recognition of this mode of synaptic communication. Sjostrand [8] observed frequent close contacts between extensions of b-type receptors (cones) onto a-type receptors (rods) in the outer plexiform layer of the guinea pig retina. Cohen [9–11] and Missotten [12] observed similar contacts, both between rods and cones and between cones and cones in several species including pigeon, human, gray squirrel, and macaque. Some of these contacts were made by telodendria extending from the terminals to distant receptors. All authors agreed that these were some sort of synaptic contact, but lacking clusters of vesicles, they did not fit the criteria for a traditional synapse. These contacts were clearly shown to be gap junctions by Raviola and Gilula [13, 14] and Witkovsky and colleagues [15].

Studies in the retina lent support to the notion that electrical synaptic communication was widespread. Early intracellular recordings of carp cones by Tomita and colleagues [16] gave hints that there might be direct electrical communication between photoreceptors. Tomita et al. observed that the spectral response curve of

red cones contained a shoulder at shorter wavelengths that did not fit the Dartnall nomogram of the red pigment, suggesting the possibility of another receptor type contributing to the response. Clear physiological evidence that there were electrotonic synaptic contacts between photoreceptors came from the work of Baylor and colleagues. They found that the receptive fields of cones were substantially larger than their diameters and further that current injected into one cone was passed to some other cones up to 40 μm away [17]. They noted that this was about the length of the telodendria observed in turtle retina [18], suggesting that these processes serve the purpose of direct electrical communication between the receptors, and implicating gap junctions as the route for electrical communication.

Concomitant with work on photoreceptors, similar, but much larger close membrane appositions were noted among axon terminals of horizontal cells [19]. O'Daly [20] found these also among the dendrites and further noted the similarity of these to electrotonic junctions observed in teleost motor neurons [6]. Negishi found that S-potentials spread through the network of horizontal cells, implying that there was direct electrical contact among them [21]. Shortly thereafter, Kaneko [22] gave a convincing demonstration that horizontal cells are coupled, showing both direct current flow between pairs and dye diffusion to neighboring horizontal cells. Internal and external horizontal cell networks were coupled independently and bipolar cells were not included in the network. Significantly, Kaneko also observed substantial variability in the coupling ratio, hinting at plasticity of the junctions between the cells.

Electrical synapses are also widespread in the inner plexiform layer neurons. Kolb and Famiglietti observed numerous gap junctions in the novel type II (AII) amacrine cell in the cat retina [23]. This amacrine cell holds a special place in the circuitry of the rod pathway in mammals. Kolb and Famiglietti [23–25] found that rod bipolar cells do not make synaptic output onto ganglion cells, as did cone bipolar cells. Instead, rod bipolar cells synapsed onto a dyad consisting of a type I and a type II amacrine cell. The type I amacrine cell made reciprocal synapses while the type II amacrine cell, in turn, made gap junctions onto cone bipolar cells as well as other type II amacrine cells. At least four types of cone bipolar cell receive these electrical synapses [26]. In this way, rod pathway signals in the rod bipolar cells are funneled into the cone pathway [27, 28].

The sheer abundance of gap junctions in the inner plexiform layer was revealed by Marc's electron micrographic studies using oblique sections of carp retina [29]. Capitalizing on the enhanced electron density of gap junctions compared to plasma membrane in the absence of uranyl acetate staining, Marc found over 100 gap junctions among neurons in the IPL. These included a variety of amacrine-to-amacrine and bipolar-to-bipolar cell gap junctions. Using systematic injections of biotinylated tracers, Vaney [30] found that many different cell types were gap-junctionally coupled in the rabbit retina. These small cationic tracers gave evidence for much more widespread coupling than had been evident from diffusion of anionic fluorescent dyes. The vast majority of amacrine cell types filled showed tracer coupling, as did several types of ganglion cell. Furthermore, there was ample evidence of heterologous coupling between certain ganglion and amacrine cells. From this variety of early studies, it was evident that every major class of retinal neuron harbored electrical synapses.

Physiological Functions of Electrical Synapses

The discovery of direct synaptic communication between photoreceptors raised questions as to what is the purpose of this communication. The most evident effect, the enlargement of the receptive field produced by electrical coupling, was accompanied by an enhancement of responses upon modest enlargement of the illuminating field for both cones [31, 32] and rods [33–36]. Thus, electrical synapses among photoreceptors served an integrating function. Indeed, this integrating function was such that toad rods reliably respond to flashes of light that bleach less than one pigment molecule in their own outer segment [37] and sum responses from an area as large as 0.5 mm^2 [38]. Other studies found this area of summation to be much smaller [35], but coupling was extensive nonetheless.

Another important function of photoreceptor electrical coupling is a substantial reduction in the variance of the photovoltage response resulting from averaging noncorrelated plasma membrane potential variations [34, 37]. This effect was elegantly demonstrated and modeled by Lamb and Simon [39], who showed that the voltage variance was proportional to the square root of the length constant for decay of the voltage signal within the photoreceptor network. For typical turtle cones with a length constant equal to about one cell-to-cell spacing, this resulted in an order of magnitude drop in variance of the voltage response compared to uncoupled cones. This large effect on noise improves the fidelity of the synaptic output of the photoreceptor by improving the signal-to-noise ratio at the synapse. This has been proposed to improve detection of contrast boundaries with only a modest amount of coupling [40]. In high-density photoreceptor arrays such as the primate fovea, cone coupling is expected to blur the neural representation of the image projected on the retina both optically [41, 42] and chromatically [43, 44]. However, the optical blur is calculated to be less than that produced by the optics of the eye [42], resulting in a net benefit in acuity.

A significant function of photoreceptor coupling is the crossing over of rod and cone pathways. In studies of turtle and amphibian photoreceptors, this is most evident as appearance of additional components in the spectral sensitivity curve of the rods, alteration in rod response kinetics, and enhancement of the ability of rods to follow high-frequency stimuli [45–48]. However, in mammalian retina, the substantially different architecture of the photoreceptors and their coupled network leads to the significant effects of rod–cone coupling being observable in the cones. Rod input leads to the appearance of high-sensitivity, slowly inactivating light responses in cones and cone-connected horizontal cells [49–51]. This rod input can also be detected in the inner retina. It can be observed in Off pathway ganglion cells by inhibition of the On pathway with metabotropic glutamate receptor agonists, with a sensitivity as low as 0.2 R*/rod/s [52]. In the AII amacrine cells, it is observed by blocking AMPA receptor input between rod bipolar cells and AII amacrine cells [53]. This signal, entering the AII via its gap junction with cone On bipolar cells, has a threshold 2 log units lower than cone threshold. Thus, rod–cone coupling provides a significant pathway for rod signals to activate inner retinal neurons, engaging both On and Off pathways.

Plasticity of Electrical Synapses

Horizontal Cells

Studies of horizontal cell electrical coupling in the 1970s revealed some significant variability in electrical coupling within the network. Results of Byzov [54] and Lamb [55] showed that electrical coupling of horizontal cell networks varied with lighting conditions and horizontal cell polarization. Application of bright background lights or hyperpolarization resulted in increased length constants. These changes could be accounted for by changes in membrane properties, without involving a change in the coupling resistance.

Studies of plasticity began in earnest when Negishi and Drujan [56] examined the effects of catecholamines on fish horizontal cell light responses. They found that high concentrations of adrenaline, noradrenaline, and dopamine reduced surround responses and enhanced center responses. This observation led to a number of studies that narrowed the catecholamine effects down to dopamine, establishing it as an important neuromodulator in retinal physiology [57–61]. Piccolino et al. [62] found that gamma-aminobutyric acid (GABA) receptor antagonists also reduced coupling in turtle horizontal cells. This effect was shortly shown also to be mediated by dopamine [63], suggesting that GABA antagonists increased release of dopamine from the dopaminergic interplexiform cells. The action of dopamine on horizontal cells elevated intracellular cyclic adenosine monophosphate (cAMP) and depended on adenylyl cyclase activity [64, 65], strongly suggesting this was a G-protein-coupled receptor-mediated effect. Piccolino and colleagues [66] showed that this effect on electrical coupling was dependent on activation of D1-type dopamine receptors, confirming this hypothesis.

Studies of isolated fish cone-driven horizontal cells and pairs of horizontal cells showed that electrical coupling was reduced by dopamine, in agreement with studies using dye coupling and receptive field measurements. D1 dopamine receptor activation drove the pathway, which required adenylyl cyclase activation and could be mimicked by application of membrane-permeant cAMP analogs [61, 67, 68]. The uncoupling further depended on activation of protein kinase A (PKA) [67], implicating a canonical dopamine D1 receptor pathway. The reduced electrical coupling resulted from a reduction in the open probability of the gap junction channels without a change in unitary conductance [69].

In addition to physiological changes of receptive field size and coupling conductance, horizontal cell gap junctions also show correlated ultrastructural changes. Kurz-Isler and Wolburg found that gap junction particle density in horizontal cells was high, showing crystalline organization, in dark-adapted goldfish retina. The particle density was reduced rapidly upon light adaptation, resulting in a less orderly array of particles [70, 71]. Baldridge [72] found this effect to be due to a response to dopamine via D1 receptors, suggesting that the reorganization of gap junction particles was related to the process of uncoupling the gap junction.

Modulation of horizontal cell coupling by D1 receptors has turned out to be a conserved feature of vertebrate retinal organization. In addition to teleost cone-driven

horizontal cells discussed above, this has also been seen for mudpuppy [73], turtle [74], and mammalian [75] horizontal cells. Curiously, the horizontal cells of the all-rod skate retina showed no such modulation, although they are very well coupled [76, 77]. In contrast, rod-driven horizontal cells in goldfish are regulated by dopamine D1 receptors in the same manner as the cone-driven horizontal cells [78].

A very interesting observation on electrical coupling in fish horizontal cells was that prolonged darkness reduced receptive field size and caused uncoupling, just as did dopamine [79, 80]. This result suggested that prolonged darkness caused dopamine release, leading to a controversy regarding whether or not dopamine is released in darkness. Measurements of dopamine and DOPAC in goldfish retina preparations following prolonged dark adaptation showed that dopamine release was low in the dark-adapted preparation and elevated in the light [81]. This is corroborated by similar results in several species indicating that dopamine release is relatively low, but steady in darkness, and enhanced by light stimuli [82–85]. While resolving the dopamine release controversy, this did not explain the uncoupling of horizontal cells by prolonged dark adaptation.

Rabbit horizontal cells also show the inverted U-shaped adaptation curve [86], with low coupling during prolonged dark adaptation, very high coupling with scotopic background illumination, and low coupling again in bright light. As in fish horizontal cells, this bright light-induced uncoupling depends on a D1 dopamine receptor. The mechanism underlying the inverted U-shaped adaptation curve has not yet been resolved, although it may be analogous to the mechanism of a similar effect seen in AII amacrine cells, which is discussed shortly.

AII Amacrine Cells

The retinal AII amacrine cell has been a mainstay for retinal neuroscience for four decades. As a lynchpin interneuron in the mammalian rod pathway [27, 28] and perhaps the most numerous single type of amacrine cell in the mammalian retina [87], it has been heavily studied. AII amacrine cells are extensively coupled by gap junctions, which has been known from the first description of the cell [23]. This extensive coupling is readily observed by diffusion of Neurobiotin tracer injected into single AII amacrine cells into large groups of neighbors [88]. Using this tracer diffusion as an assay for coupling, Hampson et al. [88] showed that AII amacrine cell coupling was regulated by dopamine in a manner very similar to that of horizontal cell coupling. In control conditions, a single injected AII amacrine cell led to the labeling of an average of 73 neighboring cells. Exogenously added dopamine as low as 10 nM reduced the coupled patch by more than half, with the effect increasing with dopamine concentration up to 10 μM. The dopamine effect was blocked by D1 receptor antagonist SCH 23390 and mimicked by D1 receptor agonist SKF 38393, revealing its dependence on D1 dopamine receptors just as in horizontal cells.

As dopamine is thought to be a light signal [89], it was presumed that the dopamine-dependent uncoupling is a light-adaptation mechanism. This was shown well

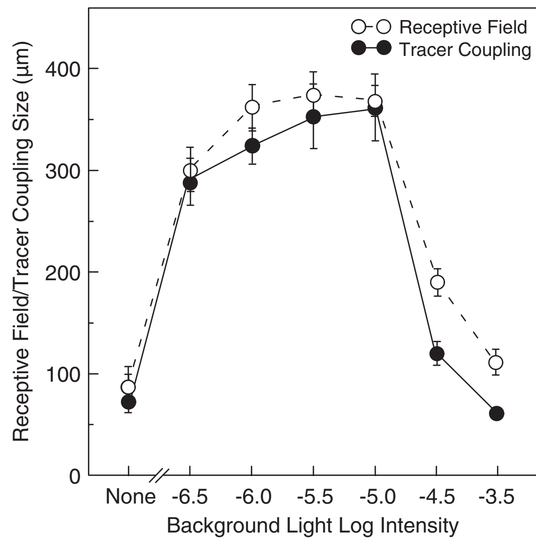

Fig. 9.1 Comparison of tracer coupling and receptive field size of rabbit AII amacrine cells injected with Neurobiotin across a range of background light intensities. Each data point represents the mean and standard error of multiple injections or recordings. Well dark-adapted retinas are represented by data point corresponding to "none" background light intensity and display very restricted coupling. Adaptation to light levels above the rod threshold result in enhanced coupling. Brighter light in the mesopic to photopic range reduces coupling again. (Reproduced with permission from Bloomfield and Volgyi 2004 [91])

by Bloomfield and colleagues [90, 91] in rabbit AII amacrine cells. Using intracellular recordings of responses to rectangular slits of light and injection of Neurobiotin tracer, they observed profound changes in coupling dependent on background lighting conditions (Fig. 9.1). In complete darkness after prolonged dark adaptation, the On center receptive field size and tracer-coupled patch of AII amacrine cells were both small, about twice the size of the dendritic arbor. With background illumination in the scotopic range, the receptive field size and the patch of tracer-coupled cells increased markedly to six to seven times the size of the dark-adapted On center receptive field. With photopic background illumination, the receptive field size and tracer-coupled patch were again reduced. In these experiments, the diameter of the receptive field and of the tracer-coupled patch were in good agreement, indicating that both measures give a valid representation of coupling among the cells. The resulting inverted U-shaped adaptation curve is strongly reminiscent of the physiological observations of horizontal cell coupling, which showed reduced coupling during prolonged dark adaptation [79, 80] and consequently have a similar inverted U-shaped adaptation curve [92]. As with horizontal cells, the uncoupling in photopic conditions is understood to result from dopamine D1 receptor activation, however, the low coupling in dark-adapted retina remained mysterious.

In a remarkable twist on the AII amacrine cell story, Mills and Massey [93] discovered that while the AII amacrine to AII amacrine cell gap junctions were regulated by dopamine and cAMP signaling as previously described, the AII amacrine to cone On bipolar cell gap junctions were not. Instead, the AII to bipolar cell gap junctions were uncoupled by nitric oxide donors and membrane-permeant cyclic guanosine monophosphate (cGMP) analogs, implicating a nitric oxide → guanylyl cyclase → cGMP → protein kinase G pathway. They further observed that the AII to bipolar cell gap junctions had a lower permeability to larger biotinylated tracers

(biotin-X-cadaverine) than did the AII–AII junctions, suggesting a molecular difference between the two types of gap junction. The stark differences between the homologous and heterologous gap junctions within the AII amacrine cell network gave a clear indication that these types of gap junctions were functionally independent.

Mills expanded on this work with detailed analysis of the regulatory properties of the AII–AII and AII–bipolar cell gap junctions [94]. This work revealed that dopamine triggered a reduction in the diffusion coefficient across the AII–bipolar cell gap junctions, but at a reduced efficacy compared to that across AII–AII gap junctions. In contrast, membrane-permeant cAMP analogs reduced both with equal efficacy. The data could be fit with a model in which gates on each side of the gap junctions were sensitive to a cAMP-based signaling pathway. D1 dopamine receptors regulated that pathway on both sides of the AII–AII gap junctions, but on only one side of the AII–bipolar cell gap junctions. Nitric oxide, on the other hand, regulated only the AII–bipolar cell gap junctions. Noteworthy is the observation that nitric oxide did not regulate coupling uniformly among the different types of cone On bipolar cell that are coupled to AII amacrine cells. Thus, nitric oxide signaling can selectively alter the relative inputs of different bipolar cells to AII amacrine cells, and vice versa.

The role of this selective regulation of coupling between AII amacrine cells and different cone On bipolar cell types can perhaps be better understood from the perspective of cone pathway function than of rod pathway function. While the AII amacrine cell is a central element of the rod pathway, in its relatively uncoupled state during photopic light adaptation it is still functional. Manookin et al. [95] showed that ganglion cells in the Off pathway receive On pathway inhibition that is derived from the transmission of On bipolar cell signals through gap junctions to AII amacrine cells. The inhibitory signals arise from glycinergic synapses onto Off ganglion cells (see [96] for ultrastructural description of this synapse). The physiological role of this pathway is most prominent as a disinhibition of the Off pathway upon light decrements and plays a fairly prominent role in ganglion cell responses to low contrast stimuli. Nitric oxide regulation of the AII–bipolar cell electrical synapses selectively regulates both the strength of this pathway and the types of bipolar cells that contribute to it.

The mechanisms of AII amacrine cell electrical synaptic plasticity have been studied in detail recently, providing the foundation upon which our understanding of the molecular mechanistic basis of electrical synaptic plasticity has been built. AII amacrine cells express Connexin 36 (Cx36) [97, 98], a connexin that was first identified in retina [99, 100] and is widely expressed throughout the central nervous system in neurons [101]. Cx36 and its fish homologue Cx35 contain PKA phosphorylation sites, and activation of PKA in a variety of expression systems results in reduction of coupling [102, 103]. This is consistent with numerous observations of electrical synaptic plasticity driven by D1 receptor activation. Indeed, it has been proposed that PKA phosphorylation of Cx36 reduces coupling, as would be predicted for activation of a dopamine D1 receptor signaling pathway [104]. However, Kothmann and colleagues, using antibodies that recognize the phosphorylated

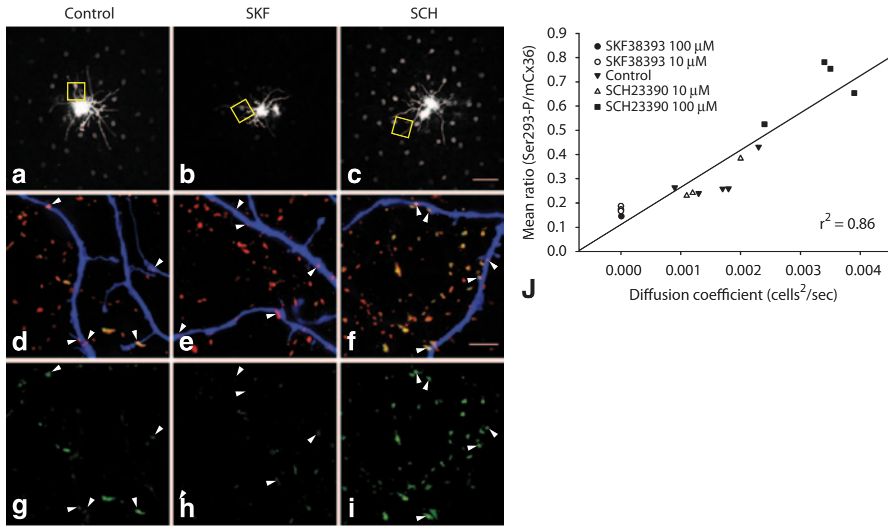

Fig. 9.2 Relationship of AII amacrine cell tracer coupling to Cx36 phosphorylation in rabbit retina. **a–c** Neurobiotin tracer coupling between AII amacrine cells is modulated by dopamine D1R signaling. D1R activation (**b**, SKF38393, 10 µM) reduced the extent of Neurobiotin diffusion relative to control (**a**). D1R antagonism (**C**, SCH23390, 100 µM) increased tracer diffusion. Yellow boxes highlight areas shown in **d** through **i** (at different focal depth). **d–f** Cx36 gap junctions, labeled with mouse anti-Cx36 antibody (*red*) and rabbit anti-phospho-Ser293 antibody (*green*), on and around the dendrites of the injected AII amacrine cell, labeled with fluorophore-conjugated Streptavidin (*blue*). The Cx36 gap junctions not on the injected cell are primarily on other AII amacrine cells. Arrowheads identify prominent Cx36 gap junctions on the injected cells. **g–i** phosphorylation of Cx36 at Ser293, a site known to regulate coupling through Cx36 gap junctions [103], is also modulated by dopamine D1R signaling. Arrowheads identify the locations of the same Cx36 gap junctions identified in (**d–f**). D1R activation (**h**, SKF38393, 10 µM) reduced Ser293-P labeling relative to control (**g**). D1R antagonism (**i**, SCH23390, 100 µM) increased Ser293-P labeling. **j** Quantification of the relationship between AII amacrine cell coupling and Cx36 phosphorylation at Ser293. The mean ratio of Ser293-P intensity to mCx36 intensity across all Cx36 gap junctions in three images per injection is plotted against the diffusion coefficient for Neurobiotin tracer transfer calculated for each injected AII amacrine cell network. The strong correlation of the data ($r^2 = 0.86$) indicates a direct relationship between AII amacrine cell coupling and Cx36 phosphorylation at Ser293. Scale bar in **c** is 50 µm; bar in **f** is 5 µm. (Reproduced with permission from Kothmann et al. 2009 [106])

regulatory sites of fish Cx35 [105], showed very clearly that D1 receptor and PKA activation actually *reduce* Cx36 phosphorylation (Fig. 9.2) [106]. This paradoxical effect was due to activation of protein phosphatase 2A by PKA activity. Kothmann et al. observed an essentially linear relationship between the phosphorylation state of Cx36, as represented by a ratio of phospho-Cx36 to total Cx36 immunostaining on each individual gap junction, and the diffusion coefficient for Neurobiotin through the network of coupled AII amacrine cells. This relationship held over a 20-fold dynamic range of coupling, representing most if not all of the physiological plasticity of the electrical synapse.

The dopamine D1 receptor-mediated dephosphorylation of Cx36 accounted for the photopic light-adaptation phase of the AII amacrine cell adaptation curve, but did not account for the low coupling observed in dark-adapted retina. However, the unexpected role of a D1 receptor-activated phosphatase did suggest a potential mechanism: that basal activity of the phosphatase could dephosphorylate the connexin in the absence of a phosphorylating drive. In subsequent experiments, Kothmann et al. showed that such a mechanism was indeed at work. Kothmann et al. [107] found that prolonged dark adaptation strongly dephosphorylated Cx36. Brief exposure to light or to metabotropic glutamate receptor antagonists, mimicking light by depolarizing On bipolar cells, triggered extensive Cx36 phosphorylation.

Similar to activity-dependent potentiation of many glutamatergic synapses in the central nervous system, the activity-dependent potentiation of AII amacrine cell electrical synapses depends on N-methyl-D-aspartate (NMDA) receptors. AII amacrine cell gap junctions are directly co-localized with nonsynaptic NMDA receptors that sense glutamate spillover from nearby On bipolar cells. Activation of the NMDA receptors by bipolar cell activity triggers CaM Kinase II phosphorylation of Cx36, opening the channels. Thus, the absence of nearby On bipolar cell activity during prolonged dark adaptation allows the system to relax to a state in which Cx36 is poorly phosphorylated and coupling is low. This molecular pathway accounts for all components of the inverted U-shaped adaptation curve of the AII amacrine cell. As noted earlier, horizontal cells also show a similar inverted U-shaped adaptation curve with regard to electrical coupling. While horizontal cells use connexins other than Cx36 [108–111], the similarity in physiological regulation suggests that there may be related signaling mechanisms controlling coupling of AII amacrine and horizontal cells.

A final insight from the work of Kothmann et al. came from images of phosphorylated Cx36 gap junctions. Kothmann et al. [106] observed that in control conditions, gap junctions separated by less than a micron on the same AII amacrine cell dendrite can exist in vastly different phosphorylation states (Fig. 9.3). These states could be driven to more uniform highly phosphorylated states by D1 receptor antagonists or more uniform poorly phosphorylated states by D1 receptor agonists. This observation reveals that the functional state of each gap junction is independently controlled. This results from an exquisitely local balance of activity-dependent phosphorylating and dopamine-dependent dephosphorylating signaling mechanisms at each gap junction.

Photoreceptors

Arguably, the most important neurons in the retina are the photoreceptors. Vision starts with these cells and all of the properties of the visual scene that can be encoded in ganglion cells are derived from photoreceptor outputs. Among vertebrates, it is a universal finding that photoreceptors are coupled to each other by electrical synapses. The numerous functions of photoreceptor electrical coupling all depend

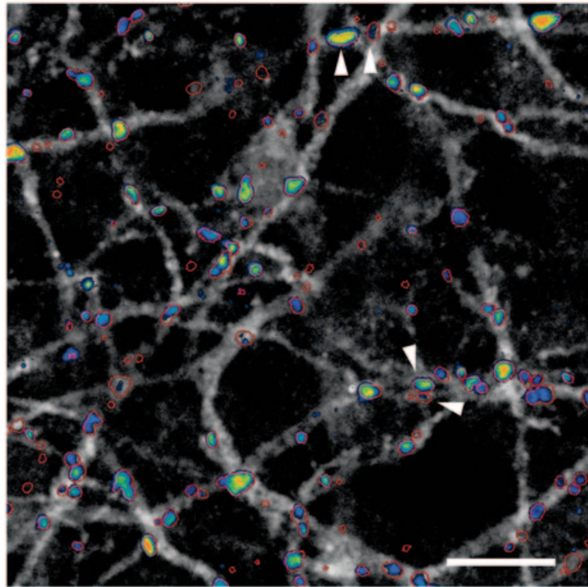

Fig. 9.3 Phosphorylation state of Cx36 gap junctions is regulated independently at each gap junction. The image shows a pseudocolor rendition of phospho-Ser293 labeling intensity on Cx36 gap junctions (*red outlines*) in the AII amacrine cell network (labeled with anti-calretinin antibody—*gray*). Some highly phosphorylated gap junctions (*orange to red colors*) are very close to weakly phosphorylated ones (*blue to black; arrowheads*) in this isolated retina from control, light-adapted conditions. Treatments with D1 receptor agonists or antagonists significantly reduce the variability of phosphorylation state between the gap junctions. Scale bar is 5 μm. (W.W. Kothmann and J. O'Brien, unpublished image from data included in Kothmann et al. 2009 [106])

quantitatively on the extent of coupling. Thus, plasticity is to be expected. However, physiological observations of plasticity in photoreceptor coupling have been less forthcoming than for other networks of neurons.

Yang and Wu [112] first demonstrated plasticity of photoreceptor coupling, finding that rod–cone coupling in salamander retina increased with background illumination. This observation was based on measurements of the increment thresholds of cones and rods to 500-nm (stimulating primarily rods) and 700-nm (stimulating primarily cones) lights as a 500-nm background light was increased. The shallower increment threshold function of rods to 700-nm stimuli than to 500-nm stimuli showed that the cone contribution steadily increased with increasing adapting background. This change in coupling was instantaneous, and is not related to modulation of the electrical synapses themselves, but rather to reduction of shunting conductances in the rod membrane as background increased.

Clear evidence that amphibian photoreceptor coupling was modulated directly was given by Krizaj et al. [48] in studies of the effects of dopamine on photoreceptor physiology and coupling. Krizaj et al. found that cone input into rods, as measured by the ability of rods to follow high-frequency sinusoidal stimuli, was substantially enhanced by D2 receptor agonist quinpirole and was eliminated by D2 receptor antagonist spiperone. Tracer-coupling measurements supported this conclusion. This effect did not extend to rods, the coupling of which remained essentially unaffected

by either quinpirole or spiperone. These results suggest that rod–cone coupling should increase with light adaptation.

In stark contrast to the results in amphibian photoreceptors, teleost and mammalian photoreceptor networks show the opposite pattern of regulation with dopamine. Wang and Mangel [113] found that responses of luminosity-type cone-driven horizontal cells in goldfish retina following prolonged dark adaptation during the night resembled those of rod-driven horizontal cells. The horizontal cells had a slow light response waveform and intensity–response functions and spectral sensitivity consistent with rod input. These features were not evident following dark adaptation in the daytime, and were quickly converted to cone-type responses by sensitizing flashes of photopic light. The appearance of rod signals in the cone-driven horizontal cells was regulated by a circadian clock, and was present only in the subjective night. One plausible explanation for this finding is that rod–cone gap junctions were open during the subjective night and shut down during the subjective day, altering the rod input into cones.

Ribelayga et al. [114] subsequently showed that the regulation of rod input into cone-driven horizontal cells was driven by dopamine through a receptor with D2-like pharmacology. Since photoreceptors have D2-like receptors while horizontal cells do not, this again strongly implicated a change in rod–cone coupling as the origin of this change in rod input. Using direct measurements of photoreceptor coupling, Ribelayga et al. [115] showed that indeed there were large changes in photoreceptor coupling driven by a circadian rhythm and dopamine D2-like receptors. Filling individual cones in goldfish retina with biocytin by iontophoresis, very few cells were labeled during subjective day, but very large patches of photoreceptors were labeled during subjective night (Fig. 9.4). Bright-light adaptation, but not dim-light adaptation during the subjective night reduced coupling to the daytime level, as did activation of D2-like dopamine receptors with quinpirole. Conversely, D2 receptor inhibition with spiperone during the subjective day enhanced coupling to the nighttime level. Both rod and cone photoreceptors were included in the coupled network.

The changes in coupling had prominent effects on cone function. Recording from cones in intact retina, they found that the cone light response threshold was nearly three orders of magnitude lower in retina during subjective night or during subjective day in the presence of spiperone (Fig. 9.5). Adaptation to bright light during the subjective night likewise elevated the response threshold by a similar amount, although dim-light adaptation did not do so. However, adaptation to scotopic background light during the subjective day was not sufficient to reduce the cone response threshold, demonstrating the potent circadian control of coupling. Finally, the receptive field size of dark-adapted cones during the subjective night was predictably larger than during the subjective day (Fig. 9.5). The potent circadian control of photoreceptor coupling was also found not to be limited to fish, but was observed in mouse photoreceptors as well [115].

The mechanisms controlling photoreceptor coupling have been studied extensively in recent years. Cone photoreceptors, and apparently rods as well, use Cx36 for their gap junctions, as do AII amacrine cells [116–118]. Using the same phospho-Cx35-specific antibodies used by Kothmann et al. [105–107], Li et al. [119]

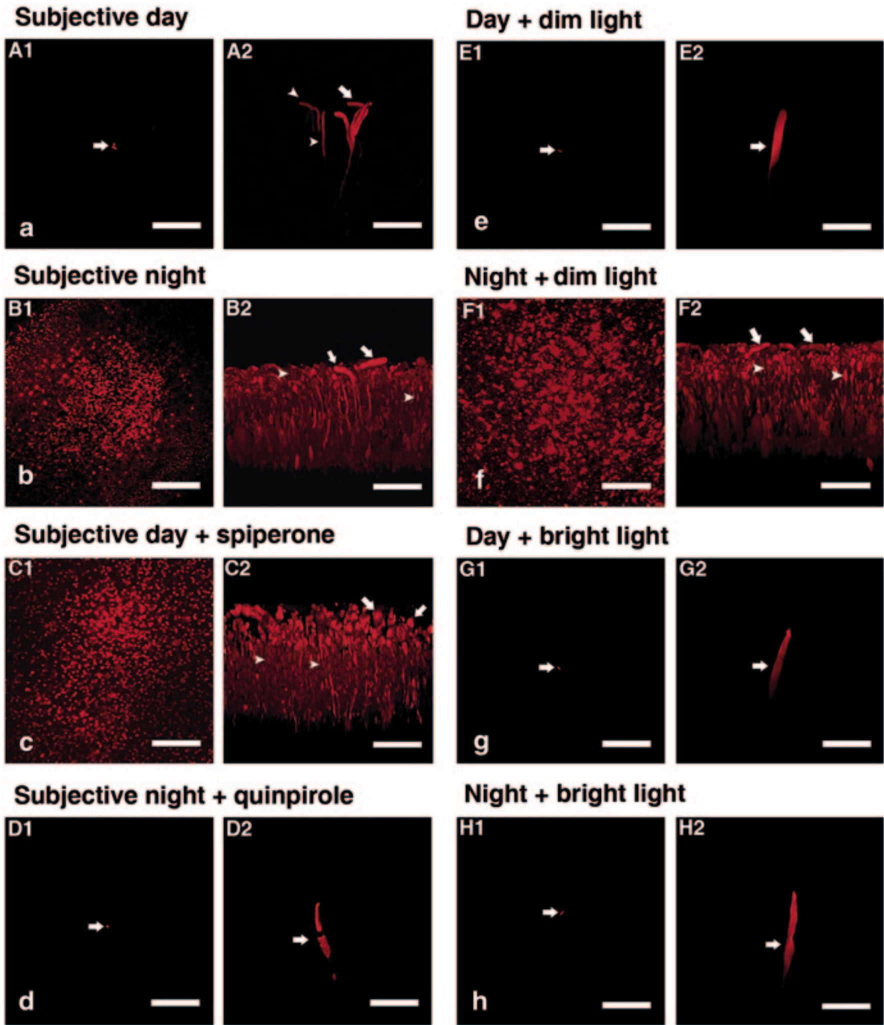

Fig. 9.4 Rod–cone tracer coupling varies with time of day. **a–h** Iontophoresis of biocytin into individual cones labeled very few cells (indicated by arrows in **a1**, **d1**, **e1**, **g1**, and **h1**) near the injected cone during the subjective day (**a**), during the subjective night in the presence of the D_2-like receptor agonist quinpirole (1 µM, **d**), and following dim-light adaptation for > 60 min in the day (**e**) and bright-light adaptation for > 60 min in the day (**g**) and night (**h**). In contrast, the tracer diffused into many rods and cones during the subjective night (**b**), during the subjective day in the presence of the D_2-like receptor antagonist spiperone (10 µM, **c**), and following dim-light adaptation for > 60 min in the night (**f**). In each of the panels **a–h**, confocal images of a whole-mount retina at the level of the rod inner segments are shown on the left, and perpendicular views of the 3D reconstruction of the photoreceptor cells from the same retina are shown on the right. Some cones (arrows) and rods (arrowheads) are indicated. Scale bars are 50 µm. (Reproduced with permission from Ribelayga et al. 2008 [115])

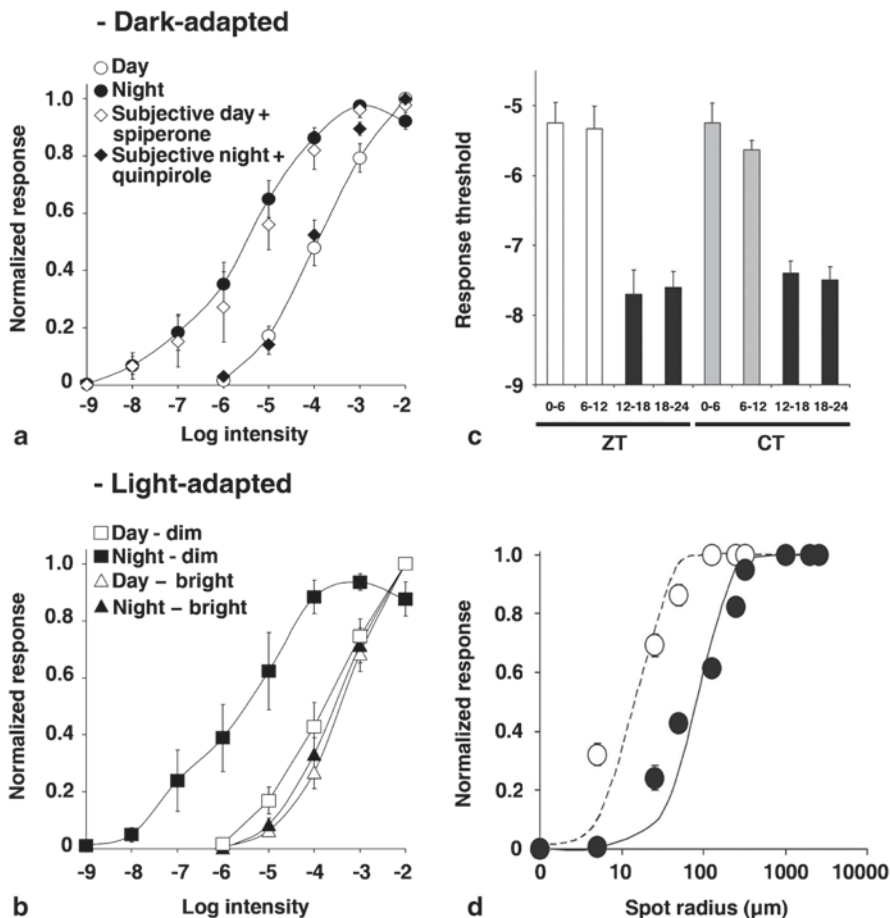

Fig. 9.5 The retinal circadian clock regulates cone receptive field size and rod input into cones. **a** Average normalized intensity–response curves of cones (one per retina) recorded under dark-adapted conditions during the day ($n=7$) and subjective day ($n=9$; *open circles*), night ($n=7$), and subjective night ($n=3$; *filled circles*), in the subjective day in the presence of spiperone (*open diamonds*, $n=5$), and in the subjective night in the presence of quinpirole (*filled diamonds*, $n=9$). **b** Intensity–response curves of cones recorded under dim light-adapted conditions during the day (*open squares*, $n=6$) and night (*filled squares*, $n=6$), and under bright light-adapted conditions in the day ($n=9$) and subjective day ($n=2$; *open triangles*) and night ($n=6$) and subjective night ($n=4$; *filled triangles*). **c** Average day/night and circadian rhythms of the cone light response threshold (i.e., intensity required to elicit a 0.5 mV response) under dark-adapted conditions. The average cone light response threshold (log intensity) was significantly higher during the day ($p<0.001$) and subjective day ($p<0.001$) than during the night and subjective night (Tukey post hoc analysis). Data points represent averages of 4–15 measurements. **d** Average normalized response amplitudes of dark-adapted cones plotted against stimulus radius for a stimulus of intensity −5 log Io. These data indicate that the receptive field size of cones is larger at night than in the day. Measurements were performed during the day (*open circles,* $n=6$) and night (*filled circles,* $n=6$). (Reproduced with permission from Ribelayga et al. 2008 [115])

found that phosphorylation of Cx35 in zebrafish photoreceptors changed in concert with changes in coupling between daytime, light-adapted conditions and nighttime dark-adapted conditions. Photoreceptor gap junctions were very poorly phosphorylated during the daytime, when coupling is low, and showed significantly enhanced phosphorylation at night. Tracer coupling was enhanced 20-fold over daytime levels, as measured by the diffusion coefficient for Neurobiotin tracer through the network, and included both cone and rod photoreceptors. Li et al. [119] found further that photoreceptor coupling and Cx35 phosphorylation depended directly on PKA activity, unlike the situation in AII amacrine cells in which PKA activity activated a phosphatase to suppress Cx36 phosphorylation and coupling [106].

Li et al. have further examined the upstream regulation of the PKA activity that controls photoreceptor coupling using a mouse model system [118]. It has long been known that the “D2-like” dopamine receptor present in mouse photoreceptors is the D4 receptor [120, 121]. Li et al. found that activation of the Gi-coupled D4 receptor by dopamine in the daytime suppressed Cx36 phosphorylation, as expected from the results of Ribelayga et al. [115]. A novel finding of this study was that the Gs-coupled adenosine A2a receptor opposed the D4 receptor effects, promoting Cx36 phosphorylation and coupling (Fig. 9.6). Extracellular adenosine is a neuromodulator that is produced in the retina at highest levels in the nighttime and in darkness [122], a pattern opposite that of dopamine [89]. Adenosine is known to modulate both rod and cone calcium currents [123–125], and several adenosine receptor types have been found in the retina [126–128]. The co-regulation of photoreceptor coupling by opposing actions of A2a receptors responding to a nighttime signal and D4 receptors responding to a daytime signal ensures tight regulatory control through the day–night cycle.

While the concept of tight regulatory control of photoreceptor coupling by opposing actions of a Gs-coupled adenosine receptor and a Gi-coupled dopamine receptor is very tidy and logical, the actual signaling pathway has turned out to be more complicated than originally envisioned. In recent work on zebrafish photoreceptor coupling, Li et al. [129] have found that the Gi-coupled adenosine A1 receptor is also involved (Fig. 9.6). Li et al. [129] found that addition of exogenous adenosine or adenosine receptor agonists in the daytime not only activated A2a receptors, enhancing Cx36 phosphorylation, but also activated A1 receptors having a dampening effect. Furthermore, the A1 receptors were tonically active in the daytime, light-adapted preparation, adding to the suppression of Cx36 phosphorylation driven by the dopamine D4 receptor. While extracellular adenosine is present at highest levels at night in dark-adapted retina, it is also present at a lower level during the day and in the light-adapted state [122]. The affinity of A1 receptors for adenosine is twofold to threefold higher than that of A2a receptors [130]. The results of Li et al. [129] suggest that the low level of extracellular adenosine present in the daytime actually reinforces the inhibitory effect of dopamine D4 receptors to keep photoreceptor coupling to a minimum in the daytime. Their results also suggest that the activity of the A2a receptor, when extracellular adenosine is high enough in dark-adapted conditions at night, is substantially more potent than that of the A1 receptor and drives enhancement of electrical coupling.

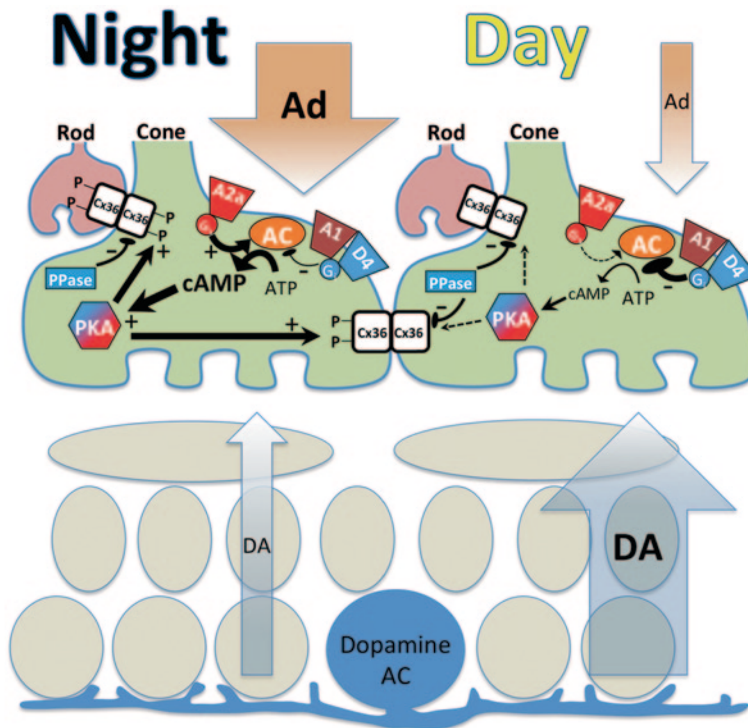

Fig. 9.6 Schematic representation of signaling mechanisms that control photoreceptor coupling. Intracellular signaling pathways control the phosphorylation of Cx36, with prominent involvement of protein kinase A to phosphorylate the connexin and an unidentified phosphatase to dephosphorylate it. Protein kinase A activity is controlled by cAMP, which in turn is controlled by adenylyl cyclase activity. Extracellular cues trigger G-protein cascades that regulate cyclase activity. Dopamine is released from the dopaminergic amacrine cell at highest levels during the circadian daytime and in response to light, but at a low level at night in darkness. It acts on D4 receptors coupled through Gi to inhibit adenylyl cyclase. Extracellular adenosine is produced primarily by enzymatic degradation of extracellular adenosine triphosphate (ATP) but its rate of re-uptake into cells is regulated by the accumulation of intracellular adenosine synthesized from AMP. Extracellular adenosine levels are highest at night and are reduced in daytime and with light adaptation. Adenosine apparently acts through both A1 and A2a receptors with opposing actions on adenylyl cyclase. The high nighttime levels of adenosine potently activate A2a receptors, which stimulate adenylyl cyclase through Gs signaling, leading to Cx36 phosphorylation and enhanced coupling. The low daytime levels of adenosine do not stimulate A2a receptors, but do activate the higher affinity A1 receptors, which signal through Gi and reinforce the D4 receptor inhibition of adenylyl cyclase. It is not known whether or not all components of this signaling mechanism are uniformly distributed among rods and the various types of cones

The G-protein-coupled receptors that impose regulatory control on photoreceptor electrical coupling are themselves subject to transcriptional control through the day–night cycle. The D4 receptor undergoes cyclic changes in transcript level through the day–night and circadian cycle [118, 131]. Similar cyclic changes in transcript level occur for adenylyl cyclase 1 (AC1), the predominant adenylyl

cyclase active in photoreceptors [118, 131, 132]. Jackson et al. [131] found that knockout of the D4 receptor gene disrupted the rhythmicity and suppressed expression of AC1. In a similar fashion, Li et al. [118] found that knockout of the A2a receptor gene disrupted the rhythmicity and suppressed expression of both AC1 and D4 receptor. Thus, it appears that signaling from the extracellular environment through G-protein-coupled receptors controls not only the plasticity of the electrical synapses but also the expression level and timing of the major components of the signaling cascade.

Finally, it is prudent to note that regulation of photoreceptor coupling is likely not uniform among photoreceptors. The report that Xenopus rod–rod coupling was not affected by dopamine receptor agonists and antagonists [48] emphasizes this point. In situ hybridization of receptor mRNA in mouse retina revealed that dopamine D4 receptor transcripts were widely distributed throughout the outer nuclear layer, while adenosine A2a receptor transcripts were apparently more strongly expressed in cones. It has also been observed that tracer-coupled networks of photoreceptors in nighttime and dark-adapted conditions contain a large proportion of cones [115, 118, 119], determined in mouse to be about twice that expected given their abundance in the retina [118]. These observations suggest that regulatory control of coupling could be stronger in cones and potentially different from that in rods. It has also made clear that cones are a central element of coupled photoreceptor networks at night in scotopic conditions, reinforcing the idea that cones are an important component of the rod pathway.

Other Retinal Neurons

In contrast to the few well-studied retinal neurons discussed above, electrical synaptic plasticity in the remaining 90% of retinal neuron types has received very little attention. Mills has made particular progress in this arena by making use of compartmental diffusion models [94, 133] to quantitatively assess tracer diffusion through heterogeneous coupled networks. In rabbit Off α ganglion cells that are homologously coupled to each other and heterologously coupled to two types of amacrine cells, Mills and colleagues [85] found that regulation differed between the gap junction types. The homologous gap junctions in the Off α ganglion cells were relatively uncoupled by activation of dopamine D2 receptors, but were unaffected by manipulation of D1 receptors. In contrast, the homologous gap junctions in the most strongly coupled type of amacrine cell ("AC1") were uncoupled by activation of D1 receptors and PKA. The ganglion cell to amacrine cell gap junctions showed more complex regulation, being sensitive to both D1 and D2 receptor activities.

Studies by Hu et al. [134] have added more complexity to the understanding of the Off α ganglion cell system. They find that, compared to retina subjected to prolonged dark adaptation, adaptation to mesopic or photopic background light dramatically *increased* the tracer-visualized field of coupled Off α ganglion cells and associated amacrine cells. This light-induced increase in coupling enhanced

the correlation of light-evoked spiking activity by nearest neighbor Off α ganglion cells. In agreement with Mills et al. [85], Hu et al. [134] found that inhibition of D2 receptors in the dark-adapted retina enhanced coupling. However, a D2 receptor agonist did not prevent the light-induced increase in coupling, which was instead blocked by a D1 antagonist. This finding suggested that the daytime level of dopamine secretion in the dark-adapted retina was sufficient to activate D2 receptors and reduce coupling among the Off α ganglion cells, but that light-evoked dopamine release recruited lower affinity D1 receptors to reverse that effect. It is difficult to reconcile these results with those of Mills et al. [85], who did not find any effect of D1 receptor agonist or antagonist on Off α ganglion cell gap junctions themselves, although they regulated the best coupled amacrine cell.

A significant difference between the two cell types is that Off α ganglion cell gap junctions behave like photoreceptor gap junctions, with adenylyl cyclase and PKA activity directly phosphorylating and opening the gap junctions, while AC1 amacrine cells behave like AII amacrine cells with adenylyl cyclase and PKA activity recruiting a phosphatase that dephosphorylates and closes the gap junctions. The study of Hu et al. [134] suggests that D1 receptors may also contribute to opening Off α ganglion cell gap junctions by activating PKA in the ganglion cells. The differences between the two study designs make it difficult to resolve this question and further research will be required to do so.

Some bipolar cell types are coupled by electrical synapses. In a number of species, several different types of both On and Off cone bipolar cells are connected by gap junctions [116, 135–137]. Rod bipolar cells have also been found to be coupled in a number of species via their dendrites [138–140] or axon terminals [29, 117]. These gap junctions are made of Cx36 [117, 140] and so are expected to display plasticity. Arai et al. [140] have found that coupling in goldfish Mb1 (rod-dominated) bipolar cells can be modulated by light adaptation. Light adaptation enhanced the coupling conductance and enabled Ca^{2+} action potentials evoked in a stimulated cell to trigger Ca^{2+} action potentials in neighboring bipolar cells. This resulted in delayed and prolonged signaling of focal stimuli to postsynaptic ganglion cells. The molecular mechanisms of this plasticity have not been explored, but are likely to involve signaling pathways similar to those discussed in previous sections.

Further Complexity in Regulation of Electrical Coupling

While nearly all reports of G-protein-coupled receptor mediated plasticity of electrical synapses in the retina concern dopamine or adenosine receptors, there is a high probability that other receptor types can also regulate coupling. A wide range of G-protein-coupled receptors are expressed in the retina and modulate synaptic processes. Two signaling mechanisms are particularly important to consider.

Cannabinoids, which act as retrograde signaling molecules in the central nervous system [141], may play a role in regulating electrical synaptic plasticity. Cannabinoids signal through G-protein-coupled receptors, with cannabinoid receptor 1 (CB1) being widely expressed in the nervous system. So far, there are no reports

of cannabinoid regulation of electrical coupling in the retina. However, the endogenous cannabinoid anadamide has been found to potently uncouple astrocyte gap junctions in a Gi/o-dependent manner [142], and CB1 activation enhances Mauthner cell electrical synaptic coupling by enhancing release of dopamine from nearby dopaminergic neurons [143]. CB1 is widely expressed in the retina of many species [144], and its activation by high concentration of agonists suppresses I_{Ca} in bipolar cells. Fan and Yazulla have observed that CB1 receptors in goldfish cones show an agonist concentration-dependent biphasic modulation of I_K, I_{Cl}, and I_{Ca} [145]. This biphasic effect was due to switching of G-protein coupling by CB1 receptors dependent on agonist concentration. The CB1 receptors signaled through Gs at low agonist concentration (<1 μM WIN 55212-2) but through Gi/o at high agonist concentration (>1 μM WIN). The Gs signaling enhanced I_K, I_{Cl}, and I_{Ca}, while Gi/o signaling at higher concentration suppressed them. D2 agonists opposed the effect of low WIN [146]. Since dopamine and adenosine receptors that regulate photoreceptor I_{Ca} also regulate coupling, it is possible that CB1 receptors are able to do so as well.

Histamine receptors are also widespread in the retina [147, 148], and respond to histamine that is released predominantly from retinopetal fibers projecting to the retina from the posterior hypothalamus [149]. Like cannabinoid receptors, these have not yet been found to modulate retinal electrical synapses. However, histamine H1 and H2 receptors have been found to modulate coupling among various populations of neurons in the supraoptic nucleus [150, 151]. H2 receptors signaled through adenylyl cyclase, but H1 receptors instead activated NO synthase, signaling through nitric oxide, guanylyl cyclase, and protein kinase G. This signaling pathway could provide a mechanism to activate the NO signaling that has been observed to uncouple horizontal cell and AII amacrine to cone On bipolar cell gap junctions.

The signaling mechanisms of G-protein-coupled receptors may be complicated by the potential for direct physical interactions between them. Adenosine A2a and dopamine D2 receptors have been found to dimerize in a variety of expression systems and neurons [152–154]. This results in allosteric modulation of receptor function. For example, activation of A2a receptor reduces the affinity of D2 receptor for dopamine and other agonists, while not affecting binding of antagonists [155–157]. Receptor–receptor heterooligomerization may also result in emergent properties not shown by either receptor. For example, dopamine D1-D2 heterooligomers have been shown to couple through Gq/11 leading to intracellular Ca^{2+} mobilization [158]. Mechanisms such as these could be relevant to any retinal electrical synapses subject to regulation by more than one type of G-protein-coupled receptor, such as photoreceptor and Off α ganglion cell electrical synapses.

Concluding Remarks

As research progresses, it has become clear that nearly all electrical synapses that are studied in detail show plasticity. This plasticity modifies neuronal connectivity, fine-tuning circuits and even imposing wholesale changes in circuitry. Cx36, the

constituent protein of the majority of electrical synapses in the retina and throughout the central nervous system, has intrinsic plasticity dependent on its phosphorylation and dephosphorylation. The well-understood forms of electrical synaptic plasticity depend on signaling mechanisms that alter this phosphorylation state, many driven by G-protein-coupled receptors. Relatively little is known about the molecular basis of plasticity in electrical synapses that do not use Cx36, but what is known suggests that the core mechanism and signaling pathways that control it are similar to those that modulate Cx36.

G-protein-coupled receptor signaling is the primary mechanism through which the plastic potential of electrical synapses is linked to the tissue environment. The biggest challenge faced by the retina is to maintain a functional state that can effectively code information across an environmental light intensity range of more than 10 orders of magnitude. The important role played by dopamine and dopamine receptors in driving light-adaptive changes in retinal physiology is well known, and changes in electrical synaptic coupling are a key part of its action. It is likely that further studies will show adenosine to have a similarly pervasive role. It is appealing to consider retinal light adaptation to be built around a daytime and light-adaptive signal (dopamine) and a nighttime and dark-adaptive signal (adenosine) that coordinately tune retinal circuits in a global manner. Dependence on two opposing signals ensures that changes in properties can be controlled with precision. It is likely that local synaptic processes and retrograde signaling from higher centers in the brain can contribute further to this plasticity to maintain optimal circuits for vision.

References

1. Bloomfield SA, Volgyi B (2009) The diverse functional roles and regulation of neuronal gap junctions in the retina. Nat Rev Neurosci 10(7):495–506. doi:10.1038/nrn2636
2. Pereda AE, Curti S, Hoge G, Cachope R, Flores CE, Rash JE (2013) Gap junction-mediated electrical transmission: regulatory mechanisms and plasticity. Biochim Biophys Acta 1828(1):134–146. doi:10.1016/j.bbamem.2012.05.026
3. Belousov AB, Fontes JD (2013) Neuronal gap junctions: making and breaking connections during development and injury. Trends Neurosci 36(4):227–236. doi:10.1016/j.tins.2012.11.001
4. Furshpan EJ, Potter DD (1957) Mechanism of nerve-impulse transmission at a crayfish synapse. Nature 180(4581):342–343. doi:10.1038/180342a0
5. Furshpan EJ, Potter DD (1959) Transmission at the giant motor synapses of the crayfish. J Physiol 145(2):289–325
6. Pappas GD, Bennett MV (1966) Specialized junctions involved in electrical transmission between neurons. Ann N Y Acad Sci 137(2):495–508. doi:10.1111/j.1749-6632.1966.tb50177.x
7. Bennett MV (1966) Physiology of electrotonic junctions. Ann N Y Acad Sci 137(2):509–539. doi:10.1111/j.1749-6632.1966.tb50178.x
8. Sjostrand FS (1958) Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstructions from serial sections. J Ultrastruct Res 2(1):122–170. doi:10.1016/S0022-5320(58)90050-9
9. Cohen AI (1963) The fine structure of the visual receptors of the pigeon. Exp Eye Res 2:88–97. doi:10.1016/S0014-4835(63)80028-7

10. Cohen AI (1964) Some observations on the fine structure of the retinal receptors of the American Gray Squirrel. Invest Ophthalmol 3:198–216
11. Cohen AI (1965) Some electron microscopic observations on inter-receptor contacts in the human and macaque retinae. J Anat 99(3):595–610
12. Missotten L, Appelmans M, Michiels J (1963) Ultrastructure of the synapses of the visual cells of the human retina. Bulletins Mem Soc Fr Ophtalmol 76:59–82
13. Raviola E, Gilula NB (1973) Gap junctions between photoreceptor cells in the vertebrate retina. Proc Natl Acad Sci U S A 70(6):1677–1681
14. Raviola E, Gilula NB (1975) Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. A freeze-fracture study in monkeys and rabbits. J Cell Biol 65(1):192–222. doi:10.1083/jcb.65.1.192
15. Witkovsky P, Shakib M, Ripps H (1974) Interreceptoral junctions in the teleost retina. Invest Ophthalmol 13(12):996–1009
16. Tomita T, Kaneko A, Murakami M, Pautler EL (1967). Spectral response curves of single cones in the carp. Vision Res 7(7):519–531. doi:10.1016/0042-6989(67)90061-2
17. Baylor DA, Fuortes MG, O'Bryan PM (1971) Receptive fields of cones in the retina of the turtle. J Physiol 214(2):265–294
18. Lasansky A (1972) Cell junctions at the outer synaptic layer of the retina. Invest Ophthalmol 11(5):265–275
19. Yamada E, Ishikawa T (1965) The fine structure of the horizontal cells in some vertebrate retinae. Cold Spring Harb Symp Quant Biol 30:383–392. doi:10.1101/SQB.1965.030.01.038
20. O'Daly JA (1967) ATPase activity at the functional contacts between retinal cells which produced S-potential. Nature 216(5122):1329–1331. doi:10.1038/2161329a0
21. Negishi K (1968) Excitation spread along horizontal and amacrine cell layers in the teleost retina. Nature 218(5136):39–40. doi:10.1038/218039a0
22. Kaneko A (1971) Electrical connexions between horizontal cells in the dogfish retina. J Physiol 213(1):95–105
23. Kolb H, Famiglietti EV (1974) Rod and cone pathways in the inner plexiform layer of cat retina. Science 186(4158):47–49. doi:10.1126/science.186.4158.47
24. Famiglietti EV Jr, Kolb H (1975) A bistratified amacrine cell and synaptic circuitry in the inner plexiform layer of the retina. Brain Res 84(2):293–300. doi:10.1016/0006-8993(75)90983-X
25. Kolb H (1979) The inner plexiform layer in the retina of the cat: electron microscopic observations. J Neurocytol 8(3):295–329. doi:10.1007/BF01236124
26. McGuire BA, Stevens JK, Sterling P (1984) Microcircuitry of bipolar cells in cat retina. J Neurosci 4(12):2920–2938
27. Strettoi E, Raviola E, Dacheux RF (1992) Synaptic connections of the narrow-field, bistratified rod amacrine cell (AII) in the rabbit retina. J Comp Neurol 325(2):152–168. doi:10.1002/cne.903250203
28. Strettoi E, Dacheux RF, Raviola E (1994) Cone bipolar cells as interneurons in the rod pathway of the rabbit retina. J Comp Neurol 347(1):139–49. doi:10.1002/cne.903470111
29. Marc RE, Liu WL, Muller JF (1988) Gap junctions in the inner plexiform layer of the goldfish retina. Vision Res 28(1):9–24. doi:10.1016/S0042-6989(88)80002-6
30. Vaney DI (1991) Many diverse types of retinal neurons show tracer coupling when injected with biocytin or neurobiotin. Neurosci Lett 125(2):187–190. doi:10.1016/0304-3940(91)90024-N
31. Fuortes MGF (1972) Responses of cones and horizontal cells in the retina of the turtle. Invest Ophthalmol Vis Sci 11(5):275–284
32. Burkhardt DA (1977) Responses and receptive-field organization of cones in perch retinas. J Neurophysiol 40(1):53–62
33. Schwartz EA (1973) Responses of single rods in the retina of the turtle. J Physiol 232(3):503–514
34. Schwartz EA (1975) Rod–rod interaction in the retina of the turtle. J Physiol 246(3):617–638
35. Copenhagen DR, Owen WG (1976) Functional characteristics of lateral interactions between rods in the retina of the snapping turtle. J Physiol 259(2):251–282

36. Werblin FS (1978) Transmission along and between rods in the tiger salamander retina. J Physiol (Lond) 280:449–470
37. Fain GL (1975) Quantum sensitivity of rods in the toad retina. Science 187(4179):838–841. doi:10.1126/science.1114328
38. Fain GL, Gold GH, Dowling JE (1976) Receptor coupling in the toad retina. Cold Spring Harb Symp Quant Biol 40:547–561. doi:10.1101/SQB.1976.040.01.051
39. Lamb TD, Simon EJ (1976) The relation between intercellular coupling and electrical noise in turtle photoreceptors. J Physiol 263(2):257–286
40. Lebedev DS, Byzov AL, Govardovskii VI (1998) Photoreceptor coupling and boundary detection. Vis Res 38(20):3161–3169. doi:10.1016/S0042-6989(98)00017-0
41. Schneeweis DM, Schnapf JL (1999) The photovoltage of macaque cone photoreceptors: adaptation, noise, and kinetics. J Neurosci 19(4):1203–1216
42. DeVries SH, Qi X, Smith R, Makous W, Sterling P (2002) Electrical coupling between mammalian cones. Curr Biol 12(22):1900–1907. doi:10.1016/S0960-9822(02)01261-7
43. Hsu A, Smith RG, Buchsbaum G, Sterling P (2000) Cost of cone coupling to trichromacy in primate fovea. J Opt Soc Am A Opt Image Sci Vis 17(3):635–640. doi:10.1364/JOSAA.17.000635
44. Hornstein EP, Verweij J, Schnapf JL (2004) Electrical coupling between red and green cones in primate retina. Nat Neurosci 7(7):745–750. doi:10.1038/nn1274, [pii] nn1274
45. Schwartz EA (1975) Cones excite rods in the retina of the turtle. J Physiol 246(3):639–651
46. Gold GH (1979) Photoreceptor coupling in retina of the toad, Bufo marinus. II. Physiology. J Neurophysiol 42(1 Pt 1):311–328
47. Wu SM, Yang XL (1988) Electrical coupling between rods and cones in the tiger salamander retina. Proc Natl Acad Sci U S A 85(1):275–278
48. Krizaj D, Gabriel R, Owen WG, Witkovsky P (1998) Dopamine D2 receptor-mediated modulation of rod-cone coupling in the Xenopus retina. J Comp Neurol 398(4):529–538. doi:10.1002/(SICI)1096-9861(19980907)398:4<529::AID-CNE5>3.0.CO;2-4
49. Nelson R (1977) Cat cones have rod input: a comparison of the response properties of cones and horizontal cell bodies in the retina of the cat. J Comp Neurol 172(1):109–135. doi:10.1002/cne.901720106
50. Dacheux RF, Raviola E (1982) Horizontal cells in the retina of the rabbit. J Neurosci 2(10):1486–1493
51. Schneeweis DM, Schnapf JL (1995) Photovoltage of rods and cones in the macaque retina. Science 268(5213):1053–1056. doi:10.1126/science.7754386
52. DeVries SH, Baylor DA (1995) An alternative pathway for signal flow from rod photoreceptors to ganglion cells in mammalian retina. Proc Natl Acad Sci U S A 92(23):10658–10662
53. Trexler EB, Li W, Massey SC (2005) Simultaneous contribution of two rod pathways to AII amacrine and cone bipolar cell light responses. J Neurophysiol 93(3):1476–1485. doi:10.1152/jn.00597.2004
54. Byzov AL (1975) Interaction between the horizontal cells of the turtle retina. Neirofiziologiia 7(3):279–286
55. Lamb TD (1976) Spatial properties of horizontal cell responses in the turtle retina. J Physiol (Lond) 263(2):239–255
56. Negishi K, Drujan BD (1978) Effects of catecholamines on the horizontal cell membrane potential in the fish retina. Sens Processes 2(4):388–395
57. Negishi K, Drujan BD (1979) Effects of catecholamines and related compounds on horizontal cells in the fish retina. J Neurosci Res 4(5–6):311–334. doi:10.1002/jnr.490040502
58. Negishi K, Drujan BD (1979) Reciprocal changes in center and surrounding S potentials of fish retina in response to dopamine. Neurochem Res 4(3):313–318. doi:10.1007/BF00963801
59. Teranishi T, Negishi K, Kato S (1983) Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina. Nature 301(5897):243–246. doi:10.1038/301243a0
60. Teranishi T, Negishi K, Kato S (1984) Regulatory effect of dopamine on spatial properties of horizontal cells in carp retina. J Neurosci 4(5):1271–1280

61. Lasater EM, Dowling JE (1985) Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells. Proc Natl Acad Sci U S A 82(9):3025–3029
62. Piccolino M, Neyton J, Witkovsky P, Gerschenfeld HM (1982) gamma-Aminobutyric acid antagonists decrease junctional communication between L-horizontal cells of the retina. Proc Natl Acad Sci U S A 79(11):3671–3675
63. Negishi K, Teranishi T, Kato S (1983) A GABA antagonist, bicuculline, exerts its uncoupling action on external horizontal cells through dopamine cells in carp retina. Neurosci Lett 37(3):261–266. doi:10.1016/0304-3940(83)90441-X
64. Van Buskirk R, Dowling JE (1981) Isolated horizontal cells from carp retina demonstrate dopamine-dependent accumulation of cyclic AMP. Proc Natl Acad Sci U S A 78(12):7825–7829
65. Dowling JE, Lasater EM, Van Buskirk R, Watling KJ (1983) Pharmacological properties of isolated fish horizontal cells. Vision Res 23(4):421–432. doi:10.1016/0042-6989(83)90089-5
66. Piccolino M, Neyton J, Gerschenfeld HM (1984) Decrease of gap junction permeability induced by dopamine and cyclic adenosine 3’:5’-monophosphate in horizontal cells of turtle retina. J Neurosci 4(10):2477–2488
67. DeVries SH, Schwartz EA (1989) Modulation of an electrical synapse between solitary pairs of catfish horizontal cells by dopamine and second messengers. J Physiol 414:351–375
68. McMahon DG (1994) Modulation of electrical synaptic transmission in zebrafish retinal horizontal cells. J Neurosci 14(3 Pt 2):1722–1734
69. McMahon DG, Knapp AG, Dowling JE (1989) Horizontal cell gap junctions: single-channel conductance and modulation by dopamine. Proc Natl Acad Sci U S A 86(19):7639–7643
70. Wolburg H, Kurz-Isler G (1985) Dynamics of gap junctions between horizontal cells in the goldfish retina. Exp Brain Res 60(2):397–401. doi:10.1007/BF00235935
71. Kurz-Isler G, Wolburg H (1986) Gap junctions between horizontal cells in the cyprinid fish alter rapidly their structure during light and dark adaptation. Neurosci Lett 67(1):7–12. doi:10.1016/0304-3940(86)90199-0
72. Baldridge WH, Ball AK, Miller RG (1987) Dopaminergic regulation of horizontal cell gap junction particle density in goldfish retina. J Comp Neurol 265(3):428–436. doi:10.1002/cne.902650310
73. Dong CJ, McReynolds JS (1991) The relationship between light, dopamine release and horizontal cell coupling in the mudpuppy retina. J Physiol (Lond) 440:291–309
74. Weiler R, Akopian A (1992) Effects of background illuminations on the receptive field size of horizontal cells in the turtle retina are mediated by dopamine. Neurosci Lett 140(1):121–124. doi:10.1016/0304-3940(92)90696-5
75. Hampson EC, Weiler R, Vaney DI (1994) pH-gated dopaminergic modulation of horizontal cell gap junctions in mammalian retina. Proc R Soc Lond B Biol Sci 255(1342):67–72. doi:10.1098/rspb.1994.0010
76. Qian H, Ripps H (1992) Receptive field properties of rod-driven horizontal cells in the skate retina. J Gen Physiol 100(3):457–478. doi:10.1085/jgp.100.3.457
77. Qian H, Malchow RP, Ripps H (1993) Gap-junctional properties of electrically coupled skate horizontal cells in culture. Vis Neurosci 10(2):287–295
78. Ribelayga C, Mangel SC (2007) Tracer coupling between fish rod horizontal cells: modulation by light and dopamine but not the retinal circadian clock. Vis Neurosci 24(3):333–344. doi:10.1017/S0952523807070319
79. Mangel SC, Dowling JE (1985) Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. Science 229(4718):1107–1109. doi:10.1126/science.4035351
80. Tornqvist K, Yang XL, Dowling JE (1988) Modulation of cone horizontal cell activity in the teleost fish retina. III. Effects of prolonged darkness and dopamine on electrical coupling between horizontal cells. J Neurosci 8(7):2279–2288
81. Weiler R, Kolbinger W, Kohler K (1989) Reduced light responsiveness of the cone pathway during prolonged darkness does not result from an increase of dopaminergic activity in the fish retina. Neurosci Lett 99(1–2):214–218. doi:10.1016/0304-3940(89)90292-9
82. Parkinson D, Rando RR (1983). Effect of light on dopamine turnover and metabolism in rabbit retina. Invest Ophthalmol Vis Sci 24(3):384–388

83. Boatright JH, Hoel MJ, Iuvone PM (1989) Stimulation of endogenous dopamine release and metabolism in amphibian retina by light- and K + -evoked depolarization. Brain Res 482(1):164–168. doi:10.1016/0006-8993(89)90555-6
84. Kirsch M, Wagner HJ (1989) Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation. Vision Res 29(2):147–154. doi:10.1016/0042-6989(89)90120-X
85. Mills SL, Xia XB, Hoshi H, Firth SI, Rice ME, Frishman LJ et al (2007) Dopaminergic modulation of tracer coupling in a ganglion-amacrine cell network. Vis Neurosci 24(4):593–608. doi:10.1017/S0952523807070575
86. Xin D, Bloomfield SA (1999) Dark- and light-induced changes in coupling between horizontal cells in mammalian retina. J Comp Neurol 405(1):75–87. doi:10.1002/(SICI)1096-9861(19990301)405:1<75::AID-CNE6>3.0.CO;2-D
87. MacNeil MA, Heussy JK, Dacheux RF, Raviola E, Masland RH (1999). The shapes and numbers of amacrine cells: matching of photofilled with Golgi-stained cells in the rabbit retina and comparison with other mammalian species. J Comp Neurol 413(2):305–326. doi:10.1002/(SICI)1096-9861(19991018)413:2<305::AID-CNE10>3.0.CO;2-E
88. Hampson EC, Vaney DI, Weiler R (1992) Dopaminergic modulation of gap junction permeability between amacrine cells in mammalian retina. J Neurosci 12(12):4911–4922
89. Witkovsky P (2004) Dopamine and retinal function. Doc Ophthalmol 108(1):17–40. doi:10.1023/B:DOOP.0000019487.88486.0a
90. Bloomfield SA, Xin D, Osborne T (1997) Light-induced modulation of coupling between AII amacrine cells in the rabbit retina. Vis Neurosci 14(3):565–576. doi:10.1017/S0952523800012220
91. Bloomfield SA, Volgyi B (2004) Function and plasticity of homologous coupling between AII amacrine cells. Vision Res 44(28):3297–3306. doi:10.1016/j.visres.2004.07.012
92. Baldridge WH (2001) Triphasic adaptation of teleost horizontal cells. Prog Brain Res 131:437–449. doi:10.1016/S0079-6123(01)31035-X
93. Mills SL, Massey SC (1995) Differential properties of two gap junctional pathways made by AII amacrine cells. Nature 377(6551):734–737. doi:10.1038/377734a0
94. Xia X, Mills S (2004) Gap junctional regulatory mechanisms in the AII amacrine cell of the rabbit retina. Vis Neurosci 21(5):791–805. doi:10.1017/S0952523804215127
95. Manookin MB, Beaudoin DL, Ernst ZR, Flagel LJ, Demb JB (2008) Disinhibition combines with excitation to extend the operating range of the OFF visual pathway in daylight. J Neurosci 28(16):4136–4150. doi:28/16/4136
96. Anderson JR, Jones BW, Watt CB, Shaw MV, Yang JH, Demill D (2011) et al Exploring the retinal connectome. Mol Vis 17:355–379
97. Mills SL, O'Brien JJ, Li W, O'Brien J, Massey SC (2001) Rod pathways in the mammalian retina use connexin36. J Comp Neurol 436(3):336–350. doi:10.1002/cne.1071
98. Feigenspan A, Teubner B, Willecke K, Weiler R (2001) Expression of neuronal connexin36 in AII amacrine cells of the mammalian retina. J Neurosci 21(1):230–239
99. O'Brien J, Al-Ubaidi MR, Ripps H. Connexin 35: a gap-junctional protein expressed preferentially in the skate retina. Mol Biol Cell 7(2):233–243. doi:10.1091/mbc.7.2.233
100. O'Brien J, Bruzzone R, White TW, Al-Ubaidi MR, Ripps H (1998) Cloning and expression of two related connexins from the perch retina define a distinct subgroup of the connexin family. J Neurosci 18(19):7625–7637
101. Condorelli DF, Parenti R, Spinella F, Salinaro AT, Belluardo N, Cardile V et al (1998) Cloning of a new gap junction gene (Cx36) highly expressed in mammalian brain neurons. Eur J Neurosci 10(3):1202–1208
102. Mitropoulou G, Bruzzone R (2003) Modulation of perch connexin35 hemi-channels by cyclic AMP requires a protein kinase A phosphorylation site. J Neurosci Res 72(2):147–157. doi:10.1002/jnr.10572
103. Ouyang X, Winbow VM, Patel LS, Burr GS, Mitchell CK, O'Brien J (2005) Protein kinase A mediates regulation of gap junctions containing connexin35 through a complex pathway. Brain Res Mol Brain Res 135(1–2):1–11. doi:10.1016/j.molbrainres.2004.10.045

104. Urschel S, Hoher T, Schubert T, Alev C, Sohl G, Worsdorfer P et al (2006) Protein kinase A mediated phosphorylation of connexin36 in mouse retina results in decreased gap junctional communication between AII amacrine cells. J Biol Chem 281(44):33163–33171. doi:10.1074/jbc.M606396200
105. Kothmann WW, Li X, Burr GS, O'Brien J (2007) Connexin 35/36 is phosphorylated at regulatory sites in the retina. Vis Neurosci 24(3):363–375 doi:10.1017/S095252380707037X
106. Kothmann WW, Massey SC, O'Brien J (2009) Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. J Neurosci 29(47):14903–14911. doi:10.1523/JNEUROSCI.3436-09.2009
107. Kothmann WW, Trexler EB, Whitaker CM, Li W, Massey SC, O'Brien J (2012) Nonsynaptic NMDA receptors mediate activity-dependent plasticity of gap junctional coupling in the AII Amacrine Cell Network. J Neurosci 32(20):6747–6759. doi:10.1523/JNEUROSCI.5087–11.2012
108. Dermietzel R, Kremer M, Paputsoglu G, Stang A, Skerrett IM, Gomes D et al (2000) Molecular and functional diversity of neural connexins in the retina. J Neurosci 20(22):8331–8343
109. Hombach S, Janssen-Bienhold U, Sohl G, Schubert T, Bussow H, Ott T et al (2004) Functional expression of connexin57 in horizontal cells of the mouse retina. Eur J Neurosci 19(10):2633–2640. doi:10.1111/j.0953-816X.2004.03360.x
110. Zoidl G, Bruzzone R, Weickert S, Kremer M, Zoidl C, Mitropoulou G (2004) et al. Molecular cloning and functional expression of ZfCx52.6: a novel connexin with hemichannel-forming properties expressed in horizontal cells of the zebrafish retina. J Biol Chem 279(4):2913–2921. doi:10.1074/jbc.M304850200
111. O'Brien JJ, Li W, Pan F, Keung J, O'Brien J, Massey SC (2006) Coupling between A-type horizontal cells is mediated by connexin 50 gap junctions in the rabbit retina. J Neurosci 26(45):11624–11636. doi:10.1523/JNEUROSCI.2296-06.2006
112. Yang XL, Wu SM (1989) Modulation of rod-cone coupling by light. Science 244(4902):352–354. doi:10.1126/science.2711185
113. Wang Y, Mangel SC (1996) A circadian clock regulates rod and cone input to fish retinal cone horizontal cells. Proc Natl Acad Sci U S A 93(10):4655–4660
114. Ribelayga C, Wang Y, Mangel SC (2002) Dopamine mediates circadian clock regulation of rod and cone input to fish retinal horizontal cells. J Physiol 544(Pt 3):801–816. doi:10.1113/jphysiol.2002.023671
115. Ribelayga C, Cao Y, Mangel SC (2008) The circadian clock in the retina controls rod-cone coupling. Neuron 59(5):790–801. doi:10.1016/j.neuron.2008.07.017
116. Feigenspan A, Janssen-Bienhold U, Hormuzdi S, Monyer H, Degen J, Sohl G et al (2004) Expression of connexin36 in cone pedicles and OFF-cone bipolar cells of the mouse retina. J Neurosci 24(13):3325–3334. doi:10.1523/JNEUROSCI.5598-03.2004
117. O'Brien J, Nguyen HB, Mills SL (2004) Cone photoreceptors in bass retina use two connexins to mediate electrical coupling. J Neurosci 24(24):5632–5642. doi:10.1523/JNEUROSCI.1248-04.2004
118. Li H, Zhang Z, Blackburn MR, Wang SW, Ribelayga CP, O'Brien J (2013) Adenosine and dopamine receptors coregulate photoreceptor coupling via gap junction phosphorylation in mouse retina. J Neurosci 33(7):3135–3150 doi:10.1523/JNEUROSCI.2807-12.2013
119. Li H, Chuang AZ, O'Brien J (2009) Photoreceptor coupling is controlled by connexin 35 phosphorylation in zebrafish retina. J Neurosci 29(48):15178–15186. doi:10.1523/JNEUROSCI.3517-09.2009
120. Cohen AI, Todd RD, Harmon S, O'Malley KL (1992) Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. Proc Natl Acad Sci U S A 89(24):12093–12097
121. Nir I, Harrison JM, Haque R, Low MJ, Grandy DK, Rubinstein M et al (2002) Dysfunctional light-evoked regulation of cAMP in photoreceptors and abnormal retinal adaptation in mice lacking dopamine D4 receptors. J Neurosci 22(6):2063–2073

122. Ribelayga C, Mangel SC (2005) A circadian clock and light/dark adaptation differentially regulate adenosine in the mammalian retina. J Neurosci 25(1):215–22. doi:10.1523/JNEUROSCI.3138-04.2005
123. Stella SL Jr, Bryson EJ, Thoreson WB (2002) A2 adenosine receptors inhibit calcium influx through L-type calcium channels in rod photoreceptors of the salamander retina. J Neurophysiol 87(1):351–360
124. Stella SL Jr, Bryson EJ, Cadetti L, Thoreson WB (2003) Endogenous adenosine reduces glutamatergic output from rods through activation of A2-like adenosine receptors. J Neurophysiol 90(1):165–174 doi:10.1152/jn.00671.200290/1/165
125. Stella SL Jr, Hu WD, Vila A, Brecha NC (2007) Adenosine inhibits voltage-dependent Ca2 + influx in cone photoreceptor terminals of the tiger salamander retina. J Neurosci Res 85(5):1126–1137. doi:10.1002/jnr.21210
126. Blazynski C (1990) Discrete distributions of adenosine receptors in mammalian retina. J Neurochem 54(2):648–655. doi:10.1111/j.1471-4159.1990.tb01920.x
127. Kvanta A, Seregard S, Sejersen S, Kull B, Fredholm BB (1997) Localization of adenosine receptor messenger RNAs in the rat eye. Exp Eye Res 65(5):595–602. doi:10.1006/exer.1996.0352
128. Zhang M, Budak MT, Lu W, Khurana TS, Zhang X, Laties AM et al (2006) Identification of the A3 adenosine receptor in rat retinal ganglion cells. Mol Vis 12:937–948
129. Li H, Chuang AZ, O'Brien J (2014) Regulation of photoreceptor gap junction phosphorylation by adenosine in zebrafish retina. Vis Neurosci 31(3):237–243. doi:10.1017/S095252381300062X
130. Fredholm BB, P. IA, Jacobson KA, Linden J, Muller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors–an update. Pharmacol Rev 63(1):1–34. doi:10.1124/pr.110.003285
131. Jackson CR, Chaurasia SS, Hwang CK, Iuvone PM (2011) Dopamine D4 receptor activation controls circadian timing of the adenylyl cyclase 1/cyclic AMP signaling system in mouse retina. Eur J Neurosci 34(1):57–64. doi:10.1111/j.1460-9568.2011.07734.x
132. Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T et al (2007) Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell 130(4):730–741. doi:10.1016/j.cell.2007.06.045
133. Mills SL, Massey SC (1998) The kinetics of tracer movement through homologous gap junctions in the rabbit retina. Vis Neurosci 15(4):765–777
134. Hu EH, Pan F, Volgyi B, Bloomfield SA (2010). Light increases the gap junctional coupling of retinal ganglion cells. J Physiol 588(Pt 21):4145–4163. doi:10.1113/jphysiol.2010.193268
135. Umino O, Maehara M, Hidaka S, Kita S, Hashimoto Y (1994) The network properties of bipolar-bipolar cell coupling in the retina of teleost fishes. Vis Neurosci 11(3):533–548. doi:10.1017/S0952523800002443
136. Jacoby RA, Marshak DW (2000) Synaptic connections of DB3 diffuse bipolar cell axons in macaque retina. J Comp Neurol 416(1):19–29. doi:10.1002/(SICI)1096-9861(20000103)416:1<6::AID-CNE2>3.0.CO;2-X
137. Mills SL (1999) Unusual coupling patterns of a cone bipolar cell in the rabbit retina. Vis Neurosci 16(6):1029–1035
138. Kujiraoka T, Saito T (1986) Electrical coupling between bipolar cells in carp retina. Proc Natl Acad Sci U S A 83(11):4063–4066
139. Cuenca N, Fernandez E, Garcia M, De Juan J (1993) Dendrites of rod dominant ON-bipolar cells are coupled by gap junctions in carp retina. Neurosci Lett 162(1–2):34–38
140. Arai I, Tanaka M, Tachibana M (2010) Active roles of electrically coupled bipolar cell network in the adult retina. J Neurosci 30(27):9260–9270 doi:10.1523/JNEUROSCI.1590-10.2010
141. Marcaggi P, Attwell D (2005) Endocannabinoid signaling depends on the spatial pattern of synapse activation. Nat Neurosci 8(6):776–781. doi:10.1038/nn1458

142. Venance L, Piomelli D, Glowinski J, Giaume C (1995) Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. Nature 376(6541):590–594. doi:10.1038/376590a0
143. Cachope R, Mackie K, Triller A, O'Brien J, Pereda AE (2007) Potentiation of electrical and chemical synaptic transmission mediated by endocannabinoids. Neuron 56(6):1034–1047. doi:10.1016/j.neuron.2007.11.014
144. Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ, Maguire G (1999) Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc Natl Acad Sci U S A 96(25):14565–14570. doi:10.1073/pnas.96.25.14565
145. Fan SF, Yazulla S (2003) Biphasic modulation of voltage-dependent currents of retinal cones by cannabinoid CB1 receptor agonist WIN 55212-2. Vis Neurosci 20(2):177–188. doi:10.1017/S095252380320208X
146. Fan SF, Yazulla S (2004) Inhibitory interaction of cannabinoid CB1 receptor and dopamine D2 receptor agonists on voltage-gated currents of goldfish cones. Vis Neurosci 21(1):69–77. doi:10.1017/S0952523804041070
147. Gastinger MJ, Barber AJ, Vardi N, Marshak DW (2006) Histamine receptors in mammalian retinas. J Comp Neurol 495(6):658–667. doi:10.1002/cne.20902
148. Vila A, Satoh H, Rangel C, Mills SL, Hoshi H, O'Brien J et al (2012) Histamine receptors of cones and horizontal cells in Old World monkey retinas. J Comp Neurol 520(3):528–543. doi:10.1002/cne.22731
149. Gastinger MJ, Tian N, Horvath T, Marshak DW (2006) Retinopetal axons in mammals: emphasis on histamine and serotonin. Curr Eye Res 31(7–8):655–667. doi:10.1080/02713680600776119
150. Hatton GI, Yang QZ (2001) Ionotropic histamine receptors and H2 receptors modulate supraoptic oxytocin neuronal excitability and dye coupling. J Neurosci 21(9):2974–2982
151. Yang QZ, Hatton GI (2002) Histamine H1-receptor modulation of inter-neuronal coupling among vasopressinergic neurons depends on nitric oxide synthase activation. Brain Res 955(1–2):115–122. doi:10.1016/S0006-8993(02)03374-7
152. Kull B, Ferre S, Arslan G, Svenningsson P, Fuxe K, Owman C et al (1999) Reciprocal interactions between adenosine A2A and dopamine D2 receptors in Chinese hamster ovary cells co-transfected with the two receptors. Biochem Pharmacol. 58(6):1035–1045
153. Canals M, Marcellino D, Fanelli F, Ciruela F, de Benedetti P, Goldberg SR et al (2003) Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J Biol Chem 278(47):46741–46749. doi:10.1074/jbc.M306451200M306451200
154. Fuxe K, Ferre S, Genedani S, Franco R, Agnati LF (2007) Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 92(1–2):210–217. doi:10.1016/j.physbeh.2007.05.034
155. Dasgupta S, Ferre S, Kull B, Hedlund PB, Finnman UB, Ahlberg S et al (1996) Adenosine A2A receptors modulate the binding characteristics of dopamine D2 receptors in stably cotransfected fibroblast cells. Eur J Pharmacol 316(2–3):325–331
156. Ferre S, von Euler G, Johansson B, Fredholm BB, Fuxe K (1991) Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci U S A 88(16):7238–7241
157. Ferraro L, Beggiato S, Tomasini MC, Fuxe K, Antonelli T, Tanganelli S (2012) A(2A)/D(2) receptor heteromerization in a model of Parkinson's disease. Focus on striatal aminoacidergic signaling. Brain Res doi:10.1016/j.brainres.2012.01.032
158. Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R et al (2007) D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci U S A 104(2):654–659. doi:10.1073/pnas.0604049104

Part III
Signaling by Photosensitive Ganglion Cells

Chapter 10
The Functional Properties of the G Protein-Coupled Receptor Melanopsin in Intrinsically Photosensitive Retinal Ganglion Cells

Novel Photoreceptors Controlling Diverse Visual Functions

Alan C. Rupp and Samer Hattar

Abstract Only slightly over a decade ago, the rods and cones in the outer retina were thought to be the exclusive photoreceptors in mammals. Since then, the discovery of an additional photopigment melanopsin (a G protein-coupled receptor, GPCR) expressed in a small subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) has expanded upon the conventional view of light detection and information flow in the retina. In this chapter, we will highlight our current understanding of the structure and function of melanopsin, the cell biology and physiology of ipRGCs, how ipRGCs are integrated into the retinal circuitry, and the role of ipRGCs in visual behaviors.

Introduction and Discovery

For decades, rods and cones were believed to be the only photoreceptors in the mammalian retina. However, the realization that mice and humans lacking rods and cones were still capable of aligning their circadian rhythms to a light/dark cycle led to the search for a new photoreceptor [1–4]. Light sensitivity has been known for nearly a half-century to be initiated by GPCR opsins that complex with a light-absorbing chromophore to form photopigments. Cloning of messenger RNA (mRNAs) from photosensitive melanophores of

S. Hattar (✉) · A. C. Rupp
Department of Biology, Johns Hopkins University, 3400 N. Charles Street,
Mudd Hall 228, Baltimore, MD 21218, USA
e-mail: shattar@jhu.edu

A. C. Rupp
e-mail: arupp2@jhu.edu

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,*
Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6_10

Xenopus laevis identified a novel opsin that was thus named melanopsin [5]. Subsequently, melanopsin was identified in the mammalian genome (termed opsin 4, or Opn4) and melanopsin expression was observed in a small subset of cells in the retinal ganglion cell layer of rodents and primates, including humans [6]. These melanopsin-expressing retinal ganglion cells were shown to be intrinsically photosensitive, capable of depolarizing in response to light in the absence of rod/cone signaling [7], and were termed intrinsically photosensitive retinal ganglion cells (ipRGCs). Their axonal projections were traced to the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL) [8], crucial mammalian circadian centers, indicating they were the likely photoreceptors mediating photoentrainment in the absence of rods and cones. In addition, ipRGCs were also found to have projections to the olivary prectectal nucleus (OPN) and superior colliculus (SC), midbrain relay stations for the pupillary light reflex and reflexive eye movements, and the lateral geniculate nucleus (LGN), the main image-processing nucleus of the thalamus (Fig. 10.1).

The initial discovery of ipRGCs was made using antibodies against melanopsin and genetic reporters driven by the melanopsin promoter. The development of more sensitive labeling methods (including Cre-based reporters and bacterial artificial chromosome transgenic mice) revealed a diversity of melanopsin-expressing RGCs [9, 10]. To date, there are five identified subtypes of ipRGCs, with distinct morphologies, light responses, and central projections: termed M1–M5 (Fig. 10.1) [10–12]. In addition, genetic characterization of ipRGCs uncovered even more diversity within the known M1–M5 subtypes: the M1 subtype can be further divided into two distinct groups by expression of the transcription factor Brn3b (i.e., Brn3b-positive and Brn3b-negative M1 ipRGCs) [13]. This diversification observed in different ipRGC subtypes likely indicates a unique contribution to distinct visual behaviors by each subtype.

This hypothesis was confirmed by the finding that the M1 subtype of ipRGCs are specifically involved in circadian photoentrainment and the pupillary light reflex [14–19], sleep induction and activity suppression in response to light (negative masking) [20–23], and even the negative effects of aberrant light on mood and learning [24]. In addition, the Brn3b-positive M1 ipRGCs are specifically required for the pupillary light reflex, while the Brn3b-negative M1 ipRGCs are sufficient for circadian photoentrainment [13]. However, the full details of these experiments are outside the scope of this chapter and have been covered extensively elsewhere (see reviews: [12, 25–27]). Instead, we will focus on the function of melanopsin, the photosensitive GPCR in ipRGCs, and the general cell biology and connectivity of ipRGCs in the retina.

Melanopsin Evolution, Protein Structure, and Chromophore

Evolution and Expression of the Melanopsin Genes

Melanopsin is a seven-pass transmembrane protein classified by sequence analysis as a GPCR of the rhodopsin-like family. Within this family, melanopsin's closest

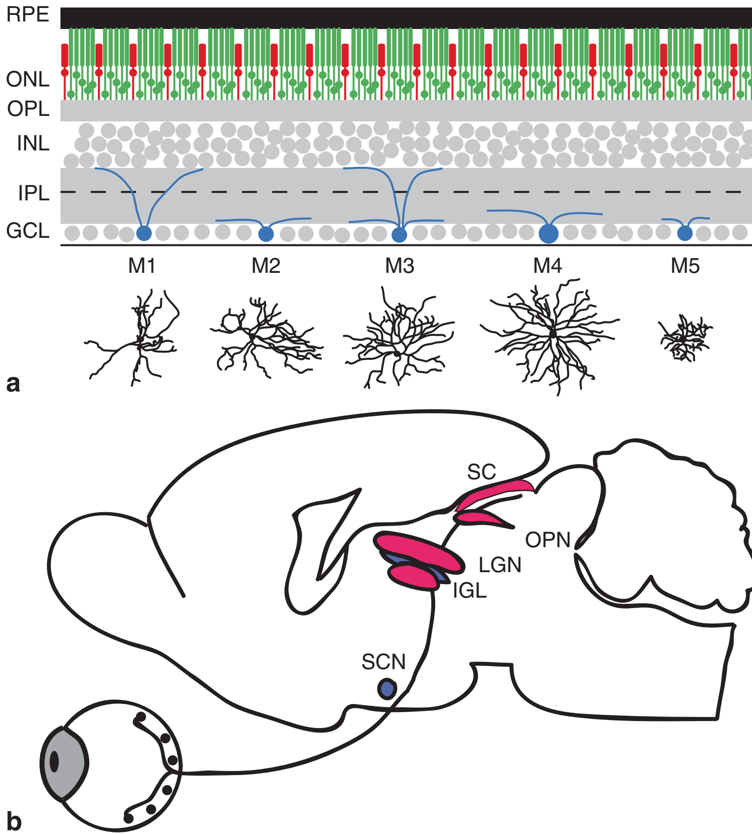

Fig. 10.1 Diversity and central projections of ipRGCs. **a** ipRGCs are located in the ganglion cell layer (*blue*) and comprise at least five distinct subtypes: M1–M5. Subtypes can be classified morphologically by soma size, dendritic morphology (including total dendrite length and dendritic field area), and stratification depth. *Above:* vertical sections of the retina in cartoon form. *Below:* Whole-mount tracings of the entire dendritic tree of actual mouse ipRGC subtypes to scale [10, 91]. **b** Cartoon depicting prominent central targets of ipRGCs, including brain areas mediating circadian functions (*SCN* and *IGL*), simple reflexive behaviors (*OPN* and *SC*), and image-forming centers (*LGN*). Areas receiving predominantly M1 innervation are colored *blue,* while areas receiving predominantly non-M1 innervation are in *red*. *SCN* suprachiasmatic nucleus, *IGL* intergeniculate leaflet, *OPN* olivary pretectal nucleus, *SC* superior colliculous, *LGN* lateral geniculate nucleus. (Model is based on [42]; please see this paper for more details on central projections)

evolutionary relatives are the invertebrate rhodopsins, and melanopsin is only distantly related to the conventional visual rod and cone opsins (Fig. 10.2) [26]. The melanopsin family itself is quite diverse. Melanopsins exist in two large families: OPN4m (for *m*ammalian) and OPN4x (for *Xenopus laevis,* the organism in which melanopsin was first identified) [28]. Nonmammalian vertebrates retain members of both families and tend to have multiple different melanopsin genes encoded in the genome. In addition, nonmammalian vertebrates express melanopsin in a variety of cell types aside from RGCs, including nonneuronal cells such as muscle and

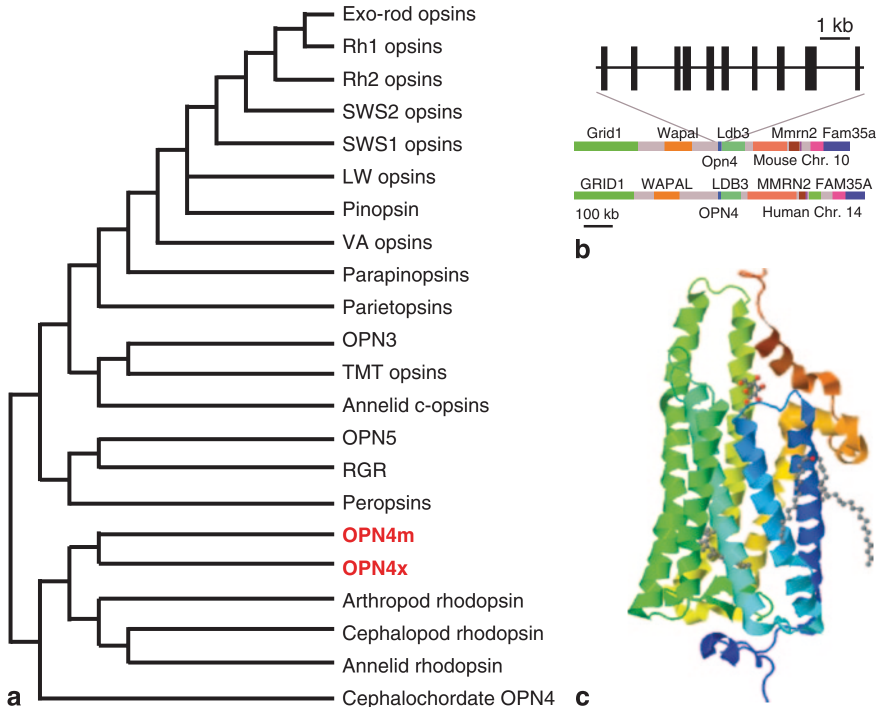

Fig. 10.2 Evolution and structure of melanopsin gene and protein. **a** Evolutionary tree of opsin genes in animals. Melanopsin families cluster with the invertebrate opsins and are distantly related to the rod and cone opsins. (Tree is adapted from [116].) Note: the branch lengths are of arbitrary distance; the tree only shows qualitative relationships. **b** Chromosomal synteny between mouse chromosome 10 and human chromosome 14, the chromosomes that contain melanopsin. The expanded view shows the gene structure of mouse melanopsin displaying exons as *black* bars. Both chromosome and gene structure are based on NCBI Gene Database gene ID 30044 and 94233. **c** Crystal structure of a C-terminal truncation of rhodopsin from the Japanese flying squid (*Todarodes pacificus*). Melanopsin structure is assumed to be highly similar, but no structure of melanopsin has been reported to date. Extracellular surface (N-terminus) is oriented downwards and intracellular surface (C-terminus) is oriented upwards. (Structure is reprinted from the RCSB Protein Data Bank (ID: 2Z73) as originally published from [117])

skin [28–30]. This broad genetic diversification and expression pattern of melanopsins in nonmammalian vertebrates indicates a role for melanopsin in a variety of light-response processes throughout the body [31]. However, though this diversity of melanopsins in nonmammalian vertebrates is interesting, the focus of this chapter will be exclusively on melanopsin in mammals.

Mammals appear to have lost the OPN4x group through chromosomal rearrangement and only have a single melanopsin gene encoded in the genome [28]. Melanopsin (Opn4) exists in the mammalian genome as 10 exons spanning about 10 kilobases of genomic space, present on chromosome 14 in mice and chromosome 10 in humans (Fig. 10.2). Although mammals have only one melanopsin gene, at least two splice isoforms of the melanopsin protein generated diversity through alternative splicing of exon 9. This alternative splicing leads to proteins with differ-

ent C termini (referred to as Opn4S for short form, the predominant form in the retina, and Opn4L for long form) [32]. The specific role of melanopsin isoforms is not clear. However, they appear to be present in distinct subtypes of ipRGCs and may have distinct light-response properties [32, 33]. In addition to sequence variability, melanopsin mRNA expression is rhythmically regulated throughout the day by light in both mammals and nonmammalian vertebrates [30, 34, 35]. In mammals, this leads to a peak in melanopsin levels at the beginning of the night and a trough during the day [34, 35]. However, ipRGC light responses differ very little from night to day [36], making the function of rhythmic expression of melanopsin unclear.

How melanopsin is cyclically regulated is completely unknown. One possible source of cyclic regulation, similar to the classical circadian clock genes [37], is at the level of gene transcription. However, virtually nothing is known about the transcription factors driving melanopsin expression. The melanopsin promoters in zebrafish have binding sites for transcriptions factors associated with rod/cone development [29]. To date, the mammalian melanopsin promoter is yet to be analyzed for transcription factor binding sites and no transcription factor mutants are known that lack melanopsin mRNA expression.

The transcription cascade that turns conventional retinal ganglion cells into photoreceptors has important evolutionary implications for the visual system. Melanopsin is among the earliest photopigments expressed in the mammalian retina, as early as embryonic day 10 in mice, long before the rod and cone opsins [38, 39]. This early expression is likely due, in part, to the fact that melanopsin is expressed in the retinal ganglion cells, which are the earliest born cells in the retina during development [40, 41]. Additionally, ipRGCs are the simplest form of photoreceptor, projecting directly to the central brain regions mediating behavior [42]. This, along with the broad identification and conservation of melanopsin in animals, makes it possible that melanopsin-expressing cells are the evolutionarily oldest photoreceptors in vertebrates and that the rest of the retina has been integrated into the existing ipRGC system.

Melanopsin Structural Characteristics

Melanopsin's unique placement in the evolution of the visual system and its distant relationship with the rod and cone opsins raise interesting questions about its structural and biophysical properties. However, very little is known about the key structural characteristics of melanopsin. One reason for this lack of knowledge is the difficulty in working with melanopsin in traditional biochemical assays. For one, melanopsin is a membrane protein present at a relatively low level in relatively few cells, precluding the ability to get large amounts of protein from the retina. Additionally, melanopsin in heterologous expression systems does not always behave like melanopsin in the retina. Until these technical hurdles are overcome, detailed knowledge of the melanopsin protein is largely limited to comparative and evolutionary analyses.

Based on its evolutionary grouping with the invertebrate opsins and the relatively well-characterized structure of squid rhodopsin, structural studies have used squid rhodopsin as a comparison (Fig. 10.2) [43]. Opsins share a variety of canonical

features, all of which are present in melanopsin: seven transmembrane domains, a DRY motif, a conserved lysine residue capable of covalently attaching to a vitamin A-based chromophore, forming a Schiff base (K337 in mouse) [44], and counter-ions to balance the charge of the Schiff base (tyrosine Y145 and/or glutamate E213) [6]. Melanopsin is also likely glycosylated as it has to be processed in the endoplasmic reticulum (ER) and contains a predicted palmitoylation site for membrane association of the C-terminus [45].

Melanopsin is a unique opsin in the mammalian retina and as a result, its phototransduction has a wide variety of features that are distinct from rod and cone phototransduction (see “Phototransduction in ipRGCs”). A more detailed knowledge of the structural and biophysical characteristics of melanopsin is crucial for understanding these specializations.

Melanopsin Chromophore Regeneration and Bistability

The photosensitive molecule in opsins is not the protein *per se,* but rather the covalently bound vitamin A-based chromophore. *Drosophila* rhodopsin is activated when light causes the chromophore 3-hydroxy-11-*cis* retinal to change to the all-*trans* form. This same process occurs in mammalian rod and cone opsins, although they use 11-*cis* retinal. Isomerization of the chromophore results in a conformational change in the opsin that promotes its guanine nucleotide exchange factor (GEF) activity, thereby activating downstream G proteins. Melanopsin also uses a vitamin A-based chromophore [46]. Specifically, melanopsin in the dark is bound to 11-*cis* retinal and the chromophore is converted to all-*trans* retinal after light activation, similar to rod and cone opsins [47].

The chromophore in isolation is broadly sensitive to light, but the protein sequence of an opsin constrains the wavelengths capable of activating the photopigment [48]. Opsins have a stereotypical absorption spectrum, differing only in the peak wavelength of activation. Melanopsin has peak activation near 480 nm, determined both electrophysiologically and behaviorally [47, 49, 50]. While mammalian rhodopsin and cone opsins enter an unstable state and dissociate from the chromophore after light activation and conversion to all-*trans, Drosophila* rhodopsin remains bound to the chromophore after its conversion to the all-*trans* state, a feature referred to as bistability. Absorption of long-wavelength light converts the all-*trans* form back to the 11-*cis* form while still bound to the opsin, thereby making it competent to be activated again.

Bistability is a general phenomenon of this clade of opsins, including cephalochordate melanopsin [51], raising the possibility that mammalian melanopsin is also bistable. In support of bistability, melanopsin is highly resistant to light or chemical bleaching [52], indicating it holds on to retinal tightly, even after intense light stimulation that would bleach rod and cone opsins. Whether melanopsin can re-isomerize all-*trans* back to 11-*cis* retinal is unclear. In support, in heterologous expression systems, melanopsin can utilize exogenous all-*trans* retinal, but only

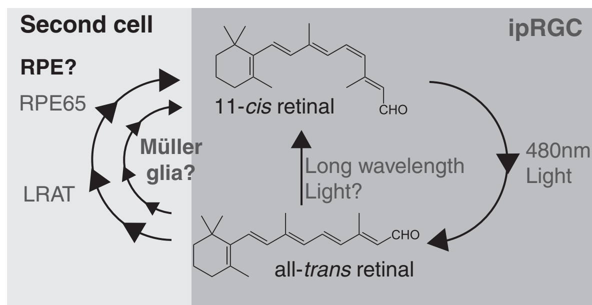

Fig. 10.3 Visual cycle of melanopsin. Melanopsin in the *dark* is bound to 11-*cis* retinal, which is isomerized to all-*trans* retinal upon light absorption. Regeneration of all-*trans* retinal to 11-*cis* requires a multistep enzymatic process, including the visual cycle enzymes *RPE65* and *LRAT,* which are present in the *RPE*. Melanopsin may also utilize an intrinsic photoisomerase capability by the absorption of long-wavelength light. Whether the Müller glia provide chromophore to melanopsin (as they do to cones) is unknown

after preexposure to long-wavelength light [53]. A prediction of a bistable photopigment is that the melanopsin population would exist in equilibrium between the 11-*cis*-bound form and the all-*trans*-bound form during broad-spectrum light stimulation as melanopsin is being concurrently activated and chromophore re-isomerized. However, after light stimulation, virtually all chromophore bound to melanopsin appears to be all-*trans* [47]. This indicates that either the rate of chromophore re-isomerization by melanopsin is significantly slower than activation or bistability is not sufficient for full regeneration of chromophore. There is currently conflict over the extent of chromophore conversion in ipRGCs. Light responses in SCN neurons downstream of ipRGCs, as well as non-image-forming behaviors, are potentiated with preexposure to long wavelength light, consistent with enhanced conversion of all-*trans* retinal back to 11-*cis* [54]. However, electrophysiological responses of ipRGCs in vitro are not potentiated with long-wavelength light [55]. Collectively, these results indicate that while melanopsin may be bistable and capable of conversion of all-*trans* to 11-*cis* retinal, there likely exists another mechanism for the regeneration of chromophore in ipRGCs (see Fig. 10.3).

What could this other mechanism be? Mammalian rods and cones rely on a different cell type to generate new 11-*cis* retinal and return it to the photoreceptors. The retinal pigment epithelium (RPE), which lines the back of the retina and is closely interleaved with the rod and cone outer segments, is their predominant source of regenerated chromophore. In addition, Müller glia provide a second route for cones to receive chromophore through a distinct chemical cycle [56, 57].

Current debate exists over whether ipRGCs utilize a second cell type for regeneration of chromophore. ipRGCs are located in the inner retina and are physically distant from the RPE, presumably giving them little access to the classical rod/cone regeneration pathway. Interestingly, ipRGC light responses at least partially require the visual cycle enzymes that are present in the RPE (RPE65 and lecithin-retinol

acyltransferase (LRAT)) [58, 59]. How these enzymes regulate the chromophore availability for melanopsin is not clear, especially because acute blockade of the RPE cycle does not affect melanopsin responses [59]. However, ipRGCs are still functional, although less sensitive, in the absence of these enzymes. One possible reason that they are still functional is due to the fact that ipRGCs appear physically close to Müller glia [60]). However, the role the Müller glia play in chromophore regeneration for ipRGCs is yet to be tested.

Another possibility is the use of a nonconventional chromophore. In the absence of RPE65, rods can utilize available 9-*cis* retinal for light detection [61]. Melanopsin in heterologous systems is also able to utilize 9-*cis* retinal [53]. Therefore, it remains possible that the residual melanopsin function in the absence of RPE65 and LRAT occurs from the availability of 9-*cis* retinal and is not a consequence of melanopsin's photoisomerase properties. In summary, the source of chromophore regeneration in ipRGCs is still a matter of debate. Melanopsin appears to utilize a variety of sources—including bistability and the RPE cycle—to generate new chromophore, although the relative importance of each is still unclear. More information on the intermediate structures of photoactivated melanopsin will help clarify this issue.

Phototransduction in ipRGCs

Physiological Properties of Melanopsin Phototransduction in ipRGCs

Non-image-forming light detection requires continuous information about the ambient light intensity of the environment, whereas image formation is best suited for rapid initiation and shutoff of the response for each absorbed photon. Melanopsin is predominantly involved in non-image-forming behaviors. Therefore, the electrophysiological properties of melanopsin phototransduction have evolved to suit these behaviors.

Rods and cones feature an incredible density of opsins and signaling components packed into specialized structures in their outer segments. ipRGCs look like conventional retinal ganglion cells with a soma, axon, and dendrites and have no such specializations [6–8]. Instead, the melanopsin protein is found throughout the soma and dendrites, with some present in the proximal axon [8]. In addition, melanopsin is expressed at a relatively low level, with 4-log units lower density of photopigment on the membrane compared to rods and cones [62]. These factors result in a low probability of photon catch by melanopsin.

In consequence, the sensitivity of ipRGCs is much lower than that of rods, the most sensitive photoreceptors in the retina, and even lower than cones. Responses of ipRGCs to millisecond flashes of light are about 6 log units less sensitive than rods and 4 less sensitive than cones [62]. However, ipRGCs have a variety of features to enhance their sensitivity in the natural environment. For instance, ipRGCs have

a resting membrane potential very close to their threshold for action potentials [11, 62]. This allows a weak signal to have a higher likelihood of being relayed to the brain. In addition, the absorption of a single photon is capable of modulating membrane conductance, indicating efficient amplification of the signal [62]. To further enhance the likelihood of firing following rare photon absorptions, ipRGCs have a long integration time (>8 s; 20 times longer than rods and >100 times longer than cones), making further depolarization possible in the event of more photon absorptions [62]. These features effectively enhance the sensitivity of ipRGCs under the prolonged light exposure that is found in the environment. In agreement, using stimuli that last for hours, ipRGC sensitivity approaches that of cones [63].

During continuous light stimuli, ipRGCs are capable of sustained firing for many minutes or hours [63]. This appears to be a unique property of ipRGCs compared to conventional RGCs. While both conventional RGCs and ipRGCs display a transient large depolarization in response to light, conventional RGCs rapidly adapt and the membrane voltage returns to baseline. ipRGCs also adapt rapidly after depolarization; however, during continuous light the light-activated current decreases to an intermediate level that allows continual spiking [11].

ipRGCs continue spiking for seconds to minutes after the stimulus ends in a dose-dependent fashion [7]. In fact, the steady state firing of ipRGCs very closely reports the ambient light intensity [7, 64]. These are ideal features for a nonimage-forming photoreceptor because it allows ipRGCs to report the average luminance of the environment continuously, even if the animal seeks cover or a cloud passes overhead.

This faithful reporting of the environmental luminance—even over many minutes and hours—implies that ipRGCs do not adapt to background light intensity or to light history in the way that rods and cones do. However, the melanopsin phototransduction pathway apparently does have an intrinsic adaptation mechanism similar to rods and cones [65]. The role light adaptation plays in ipRGCs is unclear, as it would appear to be unsuited to reporting environmental luminance. Little is known about the mechanism of adaptation, although it appears to involve both calcium-dependent and -independent components [66]. Even the response properties of light adaptation in ipRGCs can be fitted by the same equations as those used to describe rod and cone light adaptation [66], indicating they may use similar molecular mechanisms.

Phototransduction Cascade

Melanopsin is phylogenetically clustered in a group of opsins referred to as "rhabdomeric" due to their original discovery in microvillar rhabdoms of invertebrates (see Fig. 10.2). However, it is now clear that so-called rhabdomeric opsins are found in a variety of photoreceptive systems that do not have microvilli. One potentially more unifying aspect of this family of opsins is the use of a G_q or G_i/G_o signaling pathway. Indeed, *Drosophila* rhodopsin, a rhabdomeric opsin, in particular couples to a G_q [67], which triggers a phospholipase Cβ (PLCβ) signaling cascade [68].

PLCβ catalyzes the cleavage of phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacyl glycerol (DAG), inositol 1,4,5-trisphosphate (IP_3), and a proton. In a variety of organisms, IP_3 is capable of activating the IP_3 receptor on the smooth ER, releasing calcium stores and changing membrane voltage. However, *Drosophila* photoreceptor microvilli have no organelles, making this pathway impossible. A currently unclear mechanism links PLCβ activity to the opening of transient receptor potential (TRP and TRP-like or TRPL) channels and results in cation influx and depolarization, although it may be driven by PIP_2 depletion and local acidification [69] or mechanical gating [70].

Melanopsin is the only known phototransduction component that is absolutely required for intrinsic light responses in ipRGCs. Electrophysiological recordings from ipRGCs lacking melanopsin show no light-activated current or membrane voltage change [71, 72]. In addition, mice lacking rods, cones, and melanopsin have no known behavioral responses to light [73, 74]. This indicates that melanopsin is required for phototransduction in ipRGCs. Importantly, melanopsin itself is a photopigment and not just a regulator of phototransduction, as heterologous expression of melanopsin in non-light-sensitive cell types renders these cells photosensitive [49, 53, 75]. Collectively, these results indicate that melanopsin is a photosensitive molecule and the only photosensitive molecule in ipRGCs capable of activating phototransduction.

To determine the signaling components downstream of melanopsin in ipRGCs, *Drosophila* phototransduction has been used as a template. Profiling studies have identified numerous G_q, PLCβ, and TRP channel genes expressed in ipRGCs; although this is in addition to numerous other G proteins and components of other signal transduction cascades, including the IP_3 receptor [76–78]. To avoid the technical issues of scarcity of melanopsin mentioned previously, many groups have turned to expression of melanopsin in heterologous systems. However, in heterologous expression systems, melanopsin is capable of activating a variety of signaling pathways, including G_q, G_i/G_o, or G_t [44, 49, 50, 75]. Additionally, it can drive PLCβ and activate TRPC3 [49, 75] or cause release of intracellular calcium stores [53]. Therefore, to understand melanopsin function in its natural context, the phototransduction cascade must be investigated in situ.

Though phylogenetically and structurally identified as a GPCR, melanopsin and ipRGCs have also been shown experimentally to require G protein signaling for light responsiveness [53]. The G proteins downstream of melanopsin in ipRGCs are unclear, though they are widely believed to be of the G_q family. The melanopsin light response in heterologous systems or dissociated ipRGCs can be blocked with G_q-specific inhibitors, but not with G_i/G_o or G_s inhibitors [49, 53, 75, 77]; however, this could not be replicated in intact retina [77]. Which specific G_q genes melanopsin may utilize in vivo are not yet known; in mammals, the G_q family consists of four distinct genes: G_q, G_{11}, G_{14}, and G_{15}. ipRGC mRNA sequencing experiments have found expression of either a subset [78] or all of the G_q-genes [77]. However, specific knockout or knockdown approaches have not yet been performed to identify a single G_q or combination of G_q genes crucial for melanopsin phototransduction.

G_q signaling directly activates PLC, which makes PLC a good candidate for melanopsin phototransduction. All known PLCβ genes (Plcb1–4) have been found in ipRGCs, with Plcb4 being the most frequent [77]. In agreement, *Plcb4*$^{-/-}$ mice have dramatically reduced light-activated current in ipRGCs [79], indicating that PLCβ4 is likely the predominant effector molecule in melanopsin phototransduction.

How PLCβ leads to the opening of a cation channel in ipRGCs is currently unknown. PLCβ results in the production of IP_3, DAG, and a proton through the depletion of PIP_2 and generation of polyunsaturated fatty acids [80]. Application of IP_3 to ipRGCs does not result in membrane depolarization [77]. In addition, depletion of intracellular calcium stores does not block the light-mediated calcium response in ipRGCs [76, 77]. Collectively, these results rule out a role for IP_3 and the IP_3 receptor in phototransduction. The potential role of DAG is more complicated. DAG is membrane-associated and ipRGC phototransduction persists in isolated patches of membrane [77], implying DAG may be sufficient to activate the channel. However, DAG analogues do not induce a current [77]. These results leave open the question of the second messengers between PLCβ and the cation channel in ipRGCs, as they are still open for *Drosophila* photoreceptors [80].

TRP channels are downstream of PLCβ and required for invertebrate phototransduction. The mammalian genome encodes more than 30 TRP channel subunits, which can be divided into at least seven families based on sequence similarity. The mammalian family that is most similar to the Drosophila TRP and TRPL is the "canonical" family, termed TRPC. In support for TRPC in ipRGCs, TRPC antagonists block the light response of ipRGCs [76, 81, 82]. There are seven Trpc genes in the mouse and human genomes. Evidence exists for the expression of TRPC3, 6, and 7 in rodent ipRGCs [76, 81, 82], suggesting multiple TRPCs could mediate the melanopsin current. In agreement, no individual TRPC mutant is capable of abolishing the light response of ipRGCs [79, 83], though the current is reduced in *Trpc6*$^{-/-}$ mice [83]. However, *Trpc6*$^{-/-}$; *Trpc7*$^{-/-}$ double mutants display dramatically reduced light-activated current ($<1\,\%$ of wild-type) [79]. Meanwhile, *Trpc1*$^{-/-}$; *Trpc4*$^{-/-}$; *Trpc5*$^{-/-}$ triple mutant ipRGCs are completely normal [79]. These results indicate that the predominant light-responsive cation channel in ipRGCs is either redundant homomeric Trpc6 and Trpc7 channels or a heteromeric Trpc6/7 channel (Figs. 10.4 and 10.5). Ultimately, following depolarization by the TRPC channels, calcium influx occurs via L-type voltage-gated calcium channels [76, 84].

Interestingly, to date, all phototransduction mutant mice (excluding melanopsin knockout) have retained a small light-activated current in ipRGCs, indicating that there is likely at least one additional phototransduction pathway downstream of melanopsin. Its identity is currently unknown, although expression profiles and phylogenetic analyses indicate it may be a G_i/G_o pathway [26]. In addition, melanopsin in heterologous systems has been shown to promiscuously activate a variety of G proteins [44, 49, 75], indicating that while G_q may be the predominant phototransduction pathway downstream of melanopsin, ipRGCs retain redundant mechanisms for light responsiveness in its absence. To date, no behavioral tests have been

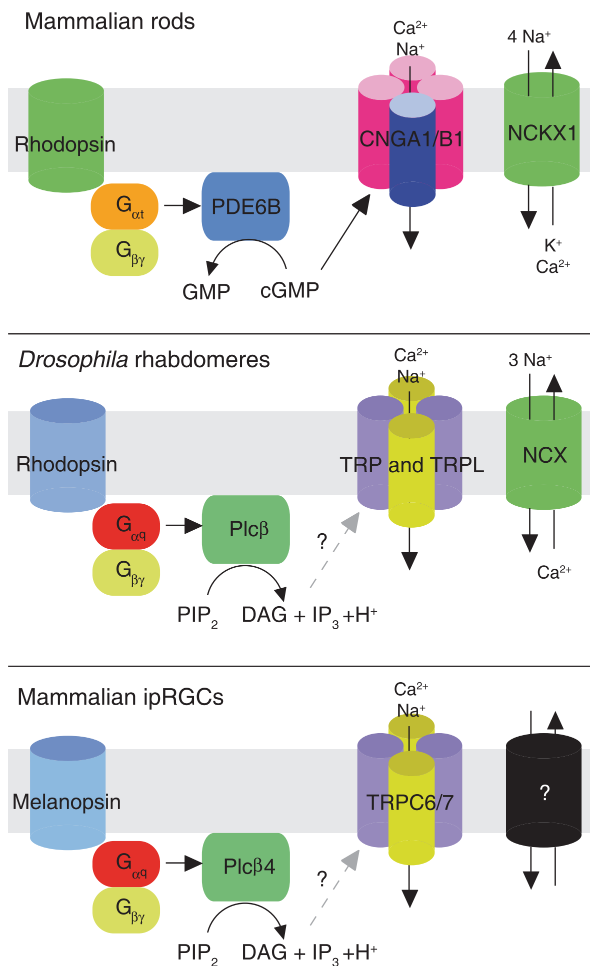

Fig. 10.4 Phototransduction in ipRGCs. Comparison of phototransduction in mammalian rods, *Drosophila* rhabdomeres, and mammalian ipRGCs. Mammalian rods use a G_i pathway and a cyclic nucleotide-gated channel to hyperpolarize in response to light. Both *Drosophila* rhodopsin and mammalian melanopsin use a G_q pathway and a transient receptor potential (*TRP*) channel to depolarize in response to light. While both mammalian rods and *Drosophila* rhabdomeres are known to utilize a calcium exchanger to reset the resting calcium concentration in the cytoplasm following phototransduction, no such channel has yet been investigated in ipRGCs

reported on G_q pathway phototransduction mutant animals. Intriguingly, mice lacking the atypical protein kinase PKCζ, not known to be involved in the G_q pathway, display behavioral deficits similar to melanopsin knockout mice [85].

Importantly, phototransduction studies have focused almost exclusively on the M1 subtype. The pathway then is presumed to be similar in the non-M1 cells. However, non-M1 ipRGCs project to image-forming regions of the brain and are more

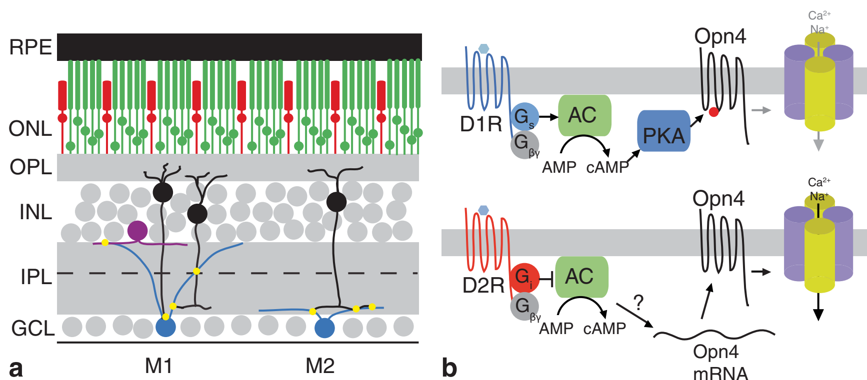

Fig. 10.5 Synaptic input to ipRGCs. **a** ipRGCs receive synaptic input from rod and cone bipolar cells, thereby allowing them to relay rod and cone light information to the brain. M1 and M2 cells receive ON bipolar cell input, although M1 cells receive nonconventional rod bipolar cell input on the soma and en passant synapses in the OFF sublamina. M1 ipRGCs are also tightly associated with the dopaminergic amacrine cells (*purple*). **b** Dopamine has multiple roles on ipRGCs. Activation of the D_1 receptor leads to a reduction in the melanopsin photocurrent through a presumed phosphorylation by *PKA*. Activation of the D_2 receptor leads to a slow enhancement of melanopsin transcription and presumably more melanopsin on the membrane and enhanced sensitivity

similar to conventional RGCs than to the M1 cells [10]. Interestingly, different ipRGC subtypes have different intrinsic light responses, differing dramatically in amplitude and kinetics [10, 11, 86]. While these differences could be attributable to variability in melanopsin levels, this may also indicate that different subtypes of ipRGCs utilize distinct phototransduction cascades. In addition, expression of melanopsin in conventional RGCs renders them photosensitive [87]. Collectively, these results suggest that melanopsin is likely to be either nonselective and hence couples to any available G protein or that melanopsin uses a generic signaling pathway that is present in a wide variety of cell types.

Regulation and Modulation of Phototransduction

All sensory systems use a variety of modulatory and regulatory proteins to ensure appropriate sensitivity and dynamics. ipRGCs are likely subject to modulation and regulation as well, although there is very little known about regulation of melanopsin and the phototransduction pathway. ipRGCs show similar light adaptation to rods and cones in response to light [65, 66] and therefore likely have similar molecular regulation.

To regulate the kinetics of phototransduction and prevent sustained G protein activation after photon absorption, rod and cone opsins are rapidly phosphorylated after light activation by a G protein-coupled receptor kinase (GRK) [88]. Phosphorylation acts to recruit arrestin to the phosphorylated opsin to prevent further G

protein activation [89]. Melanopsin is also phosphorylated in vivo following light stimulation [90]. ipRGCs express both GRK2 and GRK3 and melanopsin physically interacts with GRK2 in vitro [90], indicating a potential similar mechanism to rods and cones. However, a role for arrestin has yet to be observed in ipRGCs. In addition, melanopsin phototransduction is remarkably sustained compared to rods and cones and persists for several seconds to minutes and long after the light stimulus ends [7]. Therefore, the role of phosphorylation in melanopsin phototransduction remains unclear, although it raises interesting questions about the role of sensory adaptation in an irradiance detector.

Synaptic Input to ipRGCs

Though ipRGCs are intrinsically photosensitive, they are also retinal ganglion cells. As such, ipRGCs are integrated into the retinal circuitry and therefore can also relay indirect light information from rods and cones. ipRGCs consist of at least five distinct subtypes based on morphology and dendritic stratification, electrophysiological properties, and central projections: termed M1–M5 [9–11] (Fig. 10.1). Due to these differences, it is likely that each of these subtypes receives distinct input from the retinal circuitry.

Classically, retinal ganglion cells can be classified into two broad categories based on how they respond to light: ON cells depolarize in response to increases in light intensity, OFF cells depolarize in response to decreases in light intensity. (Note: ON–OFF cells also exist that depolarize to both.) The inner plexiform layer (IPL) contains the synaptic connections of cells in the inner nuclear layer and ganglion cell layer. Retinal ganglion cells can be presumed to be ON or OFF cells by their dendritic stratification in the IPL. ON cells have dendrites closest to RGC cell bodies in the ON sublamina, whereas OFF cells have dendrites nearest the inner nuclear layer in the OFF sublamina.

ipRGCs do not follow the stratification rules of the retina. All subtypes of ipRGCs, regardless of dendritic stratification patterns, show light-activated currents and increases in spiking in response to increases in light intensity, and are therefore ON retinal ganglion cells [9–11, 64, 86, 91]. While M2, M4, and M5 cells have dendrites exclusively in the ON sublamina, even the ipRGCs with dendrites in the OFF sublamina (M1 and M3) receive virtually no OFF input [91, 92]. This perhaps makes sense: Melanopsin effectively makes ipRGCs "ON" cells, always depolarizing in response to light increases and therefore they may want to avoid OFF input. However, the purpose of ipRGC dendrites in the OFF layer is unclear, but may be to enhance interactions with the dopaminergic cells in the retina (see "Modulation by dopamine").

The synaptic input to ipRGCs is dramatically different from the melanopsin-based light response. While melanopsin phototransduction is relatively insensitive, ipRGCs are among the most sensitive RGCs in the retina [93]. In addition, while melanopsin phototransduction is sluggish and persists after the stimulus, the synaptic input very accurately reflects the light stimulus. There is a rapid and strong

initial glutamate-driven depolarization [92], sustained firing during stimuli that can last minutes, and a rapid shutoff after the stimulus ends [11, 63]. This sustained synaptic input is also different from conventional RGCs, which largely respond with transient depolarization before relaxing to baseline. Interestingly, sustained synaptic input appears to be a general feature of ipRGCs as it is observed in all subtypes.

However, there is also substantial difference in the synaptic inputs to different ipRGC subtypes. The M1 and M2 subtypes of ipRGCs have been subject to the most study. M1 cells receive weak rod/cone input, though they have the largest intrinsic melanopsin response of any subtype [11]. This may be due to the fact that their input from the ON pathway comes from rare en passant synapses with bipolar cells in the OFF sublamina [94–96]. In addition, M1 cells appear to receive input from ON bipolar cells on their soma and proximal dendrites [97]. Intriguingly, rod bipolar cells appear to make synaptic contact with M1 cell soma [98], despite the conventional belief that rod bipolar cells never contact ganglion cells [99]. However, ipRGCs also likely receive input from the conventional rod pathway utilizing the AII amacrine cell [93]. Collectively, this sparse and unconventional input to M1 cells likely is the reason for their relatively insensitive synaptic responses. M2 cells, in turn, receive stronger rod/cone input and have conventional ON synapses [96], despite their relatively weak melanopsin response [11].

In addition to conventional ON pathway input from bipolar cells, ipRGCs also have light-mediated gamma aminobutyric acid (GABA) and glycine inputs [92]. This indicates they get substantial amacrine cell input. In fact, a viral synaptic circuit tracing study found numerous amacrine cells synaptically coupled to ipRGCs [60]. However, the role of amacrine cell input to ipRGCs is still largely unexplored.

The differential effect of the rod/cone input and melanopsin phototransduction on different ipRGC subtypes is intriguing. It is in agreement with the prediction that each subtype would be specialized for relaying specific retinal information. However, very little is known about the synaptic input and retinal pathways that impinge on the M3–M5 subtypes. These subtypes have the lowest level of melanopsin expression and presumably most rely on rod/cone input [10]. Interestingly, the M4 cells appear to be the same population as a previously studied RGC subtype: the ON alpha cell [86]. These cells have interesting electrophysiological properties, including nonlinear spatial summation, and are conventional retinal ganglion cells that likely contribute to image formation [86]. The role of melanopsin in these more conventional RGCs and how it affects pattern vision remain open questions that are crucial for understanding the role of melanopsin in vision.

Modulation by Dopamine

While synaptic input to ipRGCs has been predominantly studied in its role of relaying rod and cone light information, ipRGCs are also subject to more subtle modulation by the retinal circuitry. Dopamine is an important regulator of the mammalian retina and is released exclusively from a subpopulation of amacrine cells called dopaminergic amacrine cells (DACs). Dopamine release has a strong effect on the sensitivity and electrical coupling of rods and cones [100], resulting in changes

in retinal function throughout the day. Interestingly, ipRGCs are tightly associated with the DACs in the retina, the dendrites of ipRGCs and DACs intermingling in the IPL. This association is so strong that perturbation of the DAC dendritic stratification results in concordant perturbation of the ipRGC dendrites [101]. This close apposition also appears to be functional. Indeed, the DAC dendrites have functional release sites very close to melanopsin dendrites [102] and the two cell types appear to be synaptically coupled [60]. ipRGCs appear to express almost every identified dopamine receptor: D_1, D_2, D_4, and D_5 [103, 104], making it likely that ipRGCs are strongly influenced by the DACs and dopamine in general.

The D_1-like dopamine receptors (D_1 and D_5) activate G_s, which activates adenylyl cyclase to produce cAMP, whereas the D_2-like dopamine receptors (D_2, D_3, and D_4) have the opposite effect, activating G_i/G_o to inhibit adenylyl cyclase. Because ipRGCs express a broad array of dopamine receptors, this makes it difficult to predict the role of dopamine on ipRGCs. Acute dopamine administration activates D_1 receptors on ipRGCs, resulting in melanopsin phosphorylation by PKA and an attenuated light-activated current [81, 104, 105]. This indicates a tight balance between the rods and cones, which are capable of driving dopamine release, and ipRGCs: Greater rod/cone activation leads to more dopamine release and weaker melanopsin phototransduction.

However, dopamine is a neuromodulator capable of large-scale remodeling of retinal function across the day in addition to its acute role as a neurotransmitter [100]. These results also do not address the role of the other dopamine receptors in ipRGC physiology. Throughout the day, dopamine levels are high during the day and low at night. Conversely, melanopsin mRNA levels are low during the day and peak at the beginning of the night. This appears to be through DAC activation by rods and cones and dopamine D_2 receptor activation on ipRGCs [34, 103]. This allows another avenue through which the classical photoreceptors can regulate ipRGC sensitivity and function. However, activation of the D_1 and D_2 receptor families has opposite effects on ipRGCs (reduced photocurrent and enhanced melanopsin expression, respectively). This balance may account for the minimal differences in ipRGC sensitivity throughout the day. It is still unclear why melanopsin is subject to such dramatic regulation by dopamine.

In addition to dopamine receptors, ipRGCs express serotonin and metabotrobic GABA receptors [78]; however, their role in modulation of ipRGCs is currently unknown. Interestingly, DACs co-release GABA [106], implying ipRGCs could be regulated by the DACs in multiple ways. Presumably, many more candidate neuromodulators exist that are important for ipRGC function and regulation. We currently know very little and are only beginning to scratch the surface of how ipRGC sensitivity is set and how ipRGCs are integrated into the retinal circuitry.

Centrifugal Signaling from ipRGCs

Dopamine is known to modulate both rods and cones and circadian rhythms within the retina. As photoreceptors are involved in circadian rhythms, it is maybe

unsurprising that ipRGCs are also susceptible to dopamine regulation. However, possibly the most surprising discovery about ipRGCs to date is their ability to reciprocally affect the DACs, despite the fact that DACs are *upstream* of ipRGCs in the normal retina wiring diagram [107]. Light responses in a subpopulation of DACs can be driven by ipRGCs [107, 108], although the functional implications of this connection are not clear. However, melanopsin has been found to be important for the circadian regulation of rods and cones [109], suggesting a physiological role for ipRGC–DAC connections. This indicates that in addition to rod and cone regulation of ipRGCs through dopamine, ipRGCs utilize dopamine to regulate rod and cone visual processing. This also implies that ipRGCs can regulate *their own* sensitivity through a dopamine feedback loop. This tight network likely has profound effects on the retina throughout the day and night; however, a full appreciation of the effects are yet unknown.

Given the position of ipRGCs in the retinal ganglion cell layer, how they physically affect the DACs is of great interest. Recently, M1 ipRGCs were found to contain axon collaterals that branch from the main axon and cover broad regions of the retina [110]. The axon collaterals terminate within the IPL [110], lending possibility to the idea that they might allow ipRGCs to regulate the DACs. Collectively, these results indicate that M1 ipRGCs play a central role in the regulation of visual processing, despite not being "image-forming" RGCs themselves.

Intriguingly, ipRGC axon collaterals terminate in a variety of depths within the IPL, not just the sublamina containing the DACs, suggesting they may have broader functions. It has been previously appreciated that the presence of melanopsin and ipRGCs during early embryonic development [38, 39] allows them to regulate early light-response behaviors such as neonatal light avoidance [111]. However, the proper development and organization of the retina itself is regulated by light during development [112], leaving melanopsin and ipRGCs in a unique position to regulate development of the retina. Unexpectedly, ipRGCs modulate waves of spontaneous activity of retinal ganglion cells during development, which are important for establishing proper RGC targeting in the brain [113]. Even more remarkably, light detection by melanopsin in utero is required for the proper development of the retinal vasculature [114]. The mechanism of how melanopsin influences retinal waves and vascular development is currently unknown, although axon collaterals that branch within the eye are likely candidates. To decipher the broad roles for melanopsin and ipRGCs in development, it is crucial to identify the synaptic partners of the ipRGC axon collaterals.

Connecting the dots from ipRGCs to diverse targets within the eye and brain is crucial for understanding how these basic photoreceptors can regulate broad visual functions. The realization that ipRGCs are of central importance for the establishment of the basic setup of the retina (vascular and retinal ganglion cell targeting) lends credence to the idea that ipRGCs are evolutionarily old. ipRGCs are the first photoreceptors present in the embryo and project to "basal" regions of the brain to regulate simple functions like circadian photoentrainment and the pupillary light reflex. Melanopsin itself is an evolutionarily old photopigment, present in a wide array of animals, even those that have degenerate eyes (e.g., naked mole rat) [115].

Therefore, ipRGCs and melanopsin provide an interesting model for understanding the evolution of the visual system.

Conclusion

The relatively recent identification of melanopsin in a few hundred cells in the retinal ganglion cell layer resolved a fairly esoteric mystery in vision science: how animals recognize light to regulate non-image-forming functions like circadian photoentrainment and the pupillary light reflex in the absence of rods and cones. This was thought to be the end of the melanopsin story, as it appeared relegated to subconscious light detection and is relatively rare in the retina. However, recent work has identified vast and important functions for melanopsin far beyond non-image-forming light detection, including roles in visual perception and the proper development and maintenance of the retina. As we continue to delve deeper into the role of melanopsin in the visual system, we are likely to find even more crucial aspects of melanopsin and ipRGCs in processes long believed to be beyond the scope of these simple and rare photoreceptors.

References

1. Foster RG, Provencio I, Hudson D, Fiske S, De Grip W, Menaker M (1991) Circadian photoreception in the retinally degenerate mouse (rd/rd). J Comp Physiol A Neuroethol Sens Neural Behav Physiol 169:39–50
2. Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS et al (1995) Suppression of melatonin secretion in some blind patients by exposure to bright light. N Eng J Med 332:6–11
3. Freedman MS, Lucas RJ, Soni B, von Schantz M, Munoz M, David-Gray ZK et al (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science 284:502–504
4. Lucas RJ, Freedman MS, Munoz M, Garcia-Fernandez JM, Foster RG (1999) Regulation of the mammalian pineal by non-rod, non-cone ocular photoreceptors. Science 284:505–7
5. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD (1998) Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl AcadSci U S A 95:340–345
6. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD (2000) A novel human opsin in the inner retina. J Neurosci 20:600–605
7. Berson DM, Dunn FA, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295:1070–1073
8. Hattar S, Liao H, Takao M, Berson DM, Yau K (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295:1065–1070
9. Schmidt TM, Taniguchi K, Kofuji P (2008) Intrinsic and extrinsic light responses in melanopsin-expressing ganglion cells during mouse development. J Neurophysiol 100:371–384
10. Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen S-K, LeGates TA et al (2010) Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. Neuron 67:49–60
11. Schmidt TM, Kofuji P (2009) Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. J Neurosci 29:476–482

12. Schmidt TM, Chen S-K, Hattar S (2011) Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. Trends Neurosci 34(11):572–580
13. Chen S-K, Badea TC, Hattar S (2011) Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. Nature 476:92–95.
14. Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao H et al (2008) Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature 453:102–105
15. Hatori M, Le H, Vollmers C, Keding SR, Tanaka N, Buch T et al (2008) Inducible ablation of melanopsin-expressing retinal ganglion cells reveals their central role in non-image forming visual responses. PLoS ONE 3:e2451
16. Göz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP (2008) Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms. PLoS ONE 3:e3153
17. Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC et al (2002) Role of melanopsin in circadian responses to light. Science 298:2211–2213
18. Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB et al (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science 298:2213–2216
19. Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau K (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science 299:245–247
20. Lupi D, Oster H, Thompson S, Foster RG (2008) The acute light-induction of sleep is mediated by OPN4-based photoreception. Nat Neurosci 11:1068–1073
21. Altimus CM, Güler AD, Villa KL, McNeill DS, LeGates TA, Hattar S (2008) Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. Proc Natl Acad Sci U S A 105:19998–20003
22. Tsai JW, Hannibal J, Hagiwara G, Colas D, Ruppert E, Ruby NF et al (2009) Melanopsin as a sleep modulator: circadian gating of the direct effects of light on sleep and altered sleep homeostasis in Opn4-/- mice. PLoS Biol 7:e1000125
23. Mrosovsky N, Hattar S (2003) Impaired masking responses to light in melanopsin-knockout mice. Chronobiol Int 20:989–999
24. Legates TA, Altimus CM, Wang H, Lee H, Yang S, Zhao H et al (2012) Aberrant light directly impairs mood and learning through melanonopsin-expression neurons. Nature 491:594–598
25. Güler AD, Altimus CM, Ecker JL, Hattar S (2007) Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. Cold Spring Harb Symp Quant Biol 72:509–515
26. Peirson SN, Foster RG (2006) Melanopsin: another way of signaling light. Neuron 2006:331–339
27. Lucas RJ (2012) Mammalian inner retinal photoreception. Curr Biol 23:R125–R133
28. Bellingham J, Chaurasia SS, Melyan Z, Liu C, Cameron MA, Tarttelin EE et al (2006) Evolution of melanopsin photoreceptors: discovery and characterization of a new melanopsin in nonmammalian vertebrates. PLoS Biol 4:e254
29. Davies WIL, Zheng L, Hughes S, Tamai TK, Turton M, Halford S et al (2011) Functional diversity of melanopsins and their global expression in the teleost retina. Cell Mol Life Sci 68:4115–4132
30. Matos-Cruz V, Blasic JR, Nickle B, Robinson PR, Hattar S, Halpern ME (2011) Unexpected diversity and photoperiod dependence of the zebrafish melanopsin system. PLoS ONE 6:e25111
31. Fernandes AM, Fero K, Arrenberg AB, Bergeron SA, Driever W, Burgess HA (2012) Deep brain photoreceptors control light-seeking behavior in zebrafish larvae. Curr Biol 22:2042–2047
32. Pires SS, Hughes S, Turton M, Melyan Z, Peirson SN, Zheng L et al (2009) Differential expression of two distinct functional isoforms of melanopsin (Opn4) in the mammalian retina. J Neurosci 29:12332–12342
33. Hughes S, Welsh L, Katti C, González-Menéndez I, Turton M, Halford S et al (2012) Differential expression of melanopsin isoforms Opn4 L and Opn4S during postnatal development of the mouse retina. PLoS ONE 7:e34531

34. Sakamoto K, Liu C, Tosini G (2004) Classical photoreceptors regulate melanopsin mRNA levels in the rat retina. J Neurosci 24:9693–9697
35. Mathes A, Engel L, Holthues H, Wolloscheck T, Spessert R (2007) Daily profile in melanopsin transcripts depends on seasonal lighting conditions in the rat retina. J Neuroendocr 19:952–957
36. Weng S, Wong KY, Berson DM (2009) Circadian modulation of melanopsin-driven light response in rat ganglion-cell photoreceptors. J Biol Rhythm 24:391–402
37. Gerstner JR, Yin JCP (2010) Circadian rhythms and memory formation. Nat Rev Neurosci 11:577–588
38. Tarttelin EE, Bellingham J, Bibb LC, Foster RG, Hankins MW, Gregory-Evans K et al (2003) Expression of opsin genes early in ocular development of humans and mice. Exp Eye Res 76:393–396
39. McNeill DS, Sheely CJ, Ecker JL, Badea TC, Morhardt D, Guido W et al (2011) Development of melanopsin-based irradiance detecting circuitry. Neural Dev 6:8
40. Livesey FJ, Cepko CL (2001) Vertebrate neural cell-fate determination: lessons from the retina. Nat Rev Neurosci 2:109–118
41. Lamb TD, Collin SP, Pugh Jr EN (2007) Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. Nat Rev Neurosci 8:960–976
42. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau K et al (2006) Central projections of melanopsin- expressing retinal ganglion cells in the mouse. J Comp Neurol 349:326–349
43. Sekharan S, Wei JN, Batista VS (2012) The active site of melanopsin: the biological clock photoreceptor. J Am Chem Soc 134:19536–19539
44. Newman LA, Walker MT, Brown RL, Cronin TW, Robinson PR (2003) Melanopsin forms a functional short-wavelength photopigment. Biochemistry 42:12734–12738
45. Davies WL, Foster RG, Hankins MW (2012) Focus on molecules: melanopsin. Exp Eye Res 97:161–162
46. Fu Y, Zhong H, Wang MH, Luo D, Liao H, Maeda H et al (2005) Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. Proc Natl Acad Sci U S A 102:10339–10344
47. Walker MT, Brown RL, Cronin TW, Robinson PR (2008) Photochemistry of retinal chromophore in mouse melanopsin. Proc Natl Acad Sci U S A 105:8861–8865
48. Nathans J (1999) The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments. Neuron 24:299–312
49. Qiu X, Kumbalasiri T, Carlson SM, Wong KY, Krishna V, Provencio I et al (2005) Induction of photosensitivity by heterologous expression of melanopsin. Nature 433:745–749
50. Bailes HJ, Lucas RJ (2013) Human melanopsin forms a pigment maximally sensitive to blue light (Lmax = 479 nm) supporting activatino of Gq/11 and Gi/o signalling cascades. Proc Biol Sci 280:20122987
51. Koyanagi M, Kubokawa K, Tsukamoto H, Shichida Y, Terakita A (2005) Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. Curr Biol 15:1065–1069
52. Sexton TJ, Golczak M, Palczewski K, Van Gelder RN (2012) Melanopsin is highly resistant to light and chemical bleaching in vivo. J Biol Chem 287:20888–20897
53. Melyan Z, Tarttelin EE, Bellingham J, Lucas RJ, Hankins MW (2005) Addition of human melanopsin renders mammalian cells photoresponsive. Nature 94:741–745
54. Mure LS, Rieux C, Hattar S, Cooper HM (2007) Melanopsin-dependent nonvisual responses: evidence for photopigment bistability in vivo. J Biol Rhythm 22:411–424
55. Mawad K, Van Gelder RN (2008) Absence of long-wavelength photic potentiation of murine intrinsically photosensitive retinal ganglion cell firing in vitro. J Biol Rhythm 23:387–391
56. Wang J, Estevez ME, Cornwall MC, Kefalov VJ (2009) Intra-retinal visual cycle required for rapid and complete cone dark adaptation. Nat Neurosci 12:295–302
57. Wang J, Kefalov VJ (2009) An alternative pathway mediates the mouse and human cone visual cycle. Curr Biol 19:1665–1669

58. Batten ML, Imanishi Y, Maeda T, Tu DC, Moise AR, Bronson D et al (2004) Lecithin-retinol acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. J Biol Chem 279:10422–10432
59. Tu DC, Owens LA, Anderson L, Golczak M, Doyle SE, McCall MA et al (2006) Inner retinal photoreception independent of the visual retinoid cycle. Proc Natl Acad Sci U S A 103:10426–10431
60. Viney TJ, Balint K, Hillier D, Siegert S, Boldogkoi Z, Enquist LW et al (2007) Local retinal circuits of melanopsin-containing ganglion cells identified by transsynaptic viral tracing. Curr Biol 17:981–988
61. Fan J, Rohrer B, Moiseyev G, Ma J-X, Crouch RK (2003) Isorhodopsin rather than rhodopsin mediates rod function in RPE65 knock-out mice. Proc Natl Acad Sci U S A 100:13662–13667
62. Do MTH, Kang SH, Xue T, Zhong H, Liao H-W, Bergles DE et al (2009) Photon capture and signalling by melanopsin retinal ganglion cells. Nature 457:281–287
63. Wong KY (2012) A retinal ganglion cell that can signal irradiance continuously for 10 hours. J Neurosci 32:11478–11485
64. Dacey DM, Liao H, Peterson BB, Robinson FR, Smith VC, Pokorny J et al (2005) Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature 433:749–754
65. Wong KY, Dunn FA, Berson DM (2005) Photoreceptor adaptation in intrinsically photosensitive retinal ganglion cells. Neuron 48:1001–1010
66. Do MTH, Yau K (2013) Adaptation to steady light by intrinsically photosensitive retinal ganglion cells. Proc Natl Acad Sci U S A 110:7470–7475
67. Scott K, Becker A, Sun Y, Hardy R, Zuker CS (1995) Gqα protein function in vivo: genetic dissection of its role in photoreceptor cell physiology. Neuron 15:919–927
68. Bloomquist BT, Shortridge RD, Schneuwly S, Perdew M, Montell C, Steller H et al (1988) Isolation of a putative phospholipase C gene of Drosophila, norpA, and its role in phototransduction. Cell 54:723–733
69. Huang J, Liu C-H, Hughes SA, Postma M, Schwiening CJ, Hardie RC (2010) Activation of TRP channels by protons and phosphoinositide depletion in Drosophila photoreceptors. Curr Biol 20:189–197
70. Hardie RC, Franze K (2012) Photomechanical responses in Drosophila photoreceptors. Science 338:260–263
71. Tu DC, Zhang D, Demas J, Slutsky EB, Provencio I, Holy TE et al (2005) Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. Neuron 48:987–999
72. Schmidt TM, Kofuji P (2010) Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes. J Neurosci 30:16262–16271
73. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW et al (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424:76–81
74. Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM et al (2003) Melanopsin is required for non-image-forming photic responses in blind mice. Science 301:525–527
75. Panda S, Nayak SK, Campo B, Walker JR, Hogenesch JB, Jegla T (2005) Illumination of the melanopsin signaling pathway. Science 307:600–604
76. Hartwick ATE, Bramley JR, Yu J, Stevens KT, Allen CN, Baldridge WH et al (2007) Light-evoked calcium responses of isolated melanopsin-expressing retinal ganglion cells. J Neurosci 27:13468–13480
77. Graham DM, Wong KY, Shapiro P, Frederick C, Pattabiraman K, Berson DM (2008) Melanopsin ganglion cells use a membrane-associated rhabdomeric phototransduction cascade. J Neurophysiol 99:2522–2532
78. Siegert S, Cabuy E, Scherf BG, Kohler H, Panda S, Le Y-Z et al (2012) Transcriptional code and disease map for adult retinal cell types. Nat Neurosci 15(3):487–495
79. Xue T, Do MTH, Riccio A, Jiang Z, Hsieh J, Wang HC et al (2011) Melanopsin signalling in mammalian iris and retina. Nature 479:67–73

80. Montell C (2012) Drosophila visual transduction. Trends Neurosci 35:356–363
81. Warren EJ, Allen CN, Brown RL, Robinson DW (2006) The light-activated signaling pathway in SCN-projecting rat retinal ganglion cells. Eur J Neurosci 23:2477–2487
82. Sekaran S, Lall GS, Ralphs KL, Wolstenholme AJ, Lucas RJ, Foster RG et al (2007) 2-aminoethoxydiphenylborane is an acute inhibitor of directly photosensitive retinal ganglion cell activity in vitro and in vivo. J Neurosci 27:3981–3986
83. Perez-Leighton CE, Schmidt TM, Abramowitz J, Birnbaumer L, Kofuji P (2011) Intrinsic phototransduction persists in melanopsin-expressing ganglion cells lacking diacylglycerol-sensitive TRPC subunits. Eur J Neurosci 7:1–12
84. Sekaran S, Foster RG, Lucas RJ, Hankins MW (2003) Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. Curr Biol 13:1290–1298
85. Peirson SN, Oster H, Jones SL, Leitges M, Hankins MW, Foster RG (2007) Microarray analysis and functional genomics identify novel components of melanopsin signaling. Curr Biol 17:1363–1372
86. Estevez ME, Fogerson PM, Ilardi MC, Borghuis BG, Chan E, Weng S et al (2012) Form and function of the M4 cell, an intrinsically photosensitive retinal ganglion cell type contributing to geniculocortical vision. J Neurosci 32:13608–13620
87. Lin B, Koizumi A, Tanaka N, Panda S, Masland RH (2008) Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. Proc Natl Acad Sci U S A 105:16009–16014
88. Wilden U, Hall SW, Kühn H (1986) Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. Proc Natl Acad Sci U S A 83:1174–1178
89. Nikonov SS, Brown BM, Davis JA, Zuniga FI, Bragin A, Pugh EN et al (2008) Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 59:462–474
90. Blasic JR, Brown RL, Robinson PR (2012) Light-dependent phosphorylation of the carboxy tail of mouse melanopsin. Cell Mol Life Sci 69:1551–1562
91. Schmidt TM, Kofuji P (2010) Structure and function of bistratified intrinsically, photosensitive retinal ganglion cells in the mouse. J Comp Neurol 1504:1492–1504
92. Wong KY, Dunn FA, Graham DM, Berson DM (2007) Synaptic influences on rat ganglion-cell photoreceptors. J Physiol 582:279–296
93. Weng S, Estevez ME, Berson DM (2013) Mouse ganglion-cell photoreceptors are driven by the most sensitive rod pathway and by both types of cones. Barnes S (ed) PLoS ONE 8:e66480
94. Dumitrescu ON, Pucci FG, Wong KY, Berson DM (2009) Ectopic retinal ON bipolar cell synapses in the OFF inner plexiform layer: contacts with dopaminergic amacrine cells and melanopsin ganglion cells. J Comp Neurol 517:226–244
95. Hoshi H, Liu W-L, Massey SC, Mills SL (2009) ON inputs to the OFF layer: bipolar cells that break the stratification rules of the retina. J Neurosci 29:8875–8883
96. Grünert U, Jusuf PR, Lee SCS, Nguyen DT (2011) Bipolar input to melanopsin containing ganglion cells in primate retina. Vis Neurosci 28:39–50
97. Belenky MA, Smeraski CA, Provencio I, Sollars PJ, Pickard GE (2003) Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. J Comp Neurol 460:380–393
98. Østergaard J, Hannibal J, Fahrenkrug J (2007) Synaptic contact between melanopsin-containing retinal ganglion cells and rod bipolar cells. Invest Ophthalmol Vis Sci 48:3812–3820
99. Kolb H, Famiglietti Jr EV (1974) Rod and cone pathways in the inner plexiform layer of cat retina. Science 186:47–49
100. Ribelayga C, Cao Y, Mangel SC (2008) The circadian clock in the retina controls rod-cone coupling. Neuron 59:790–801
101. Matsuoka RL, Nguyen-Ba-Charvet KT, Parray A, Badea TC, Chédotal A, Kolodkin AL (2011) Transmembrane semaphorin signalling controls laminar stratification in the mammalian retina. Nature 470:259–263
102. Vugler AA, Redgrave P, Semo M, Lawrence J, Greenwood J, Coffey PJ (2007) Dopamine neurones form a discrete plexus with melanopsin cells in normal and degenerating retina. Exp Neurol 205:26–35

103. Sakamoto K, Liu C, Kasamatsu M, Pozdeyev NV, Iuvone PM, Tosini G (2005) Dopamine regulates melanopsin mRNA expression in intrinsically photosensitive retinal ganglion cells. Eur J Neurosci 22:3129–3136
104. Van Hook MJ, Wong KY, Berson DM (2012) Dopaminergic modulation of ganglion-cell photoreceptors in rat. Eur J Neurosci 35:507–518
105. Blasic JR, Brown RL, Robinson PR (2012) Phosphorylation of mouse melanopsin by protein kinase A. PLoS ONE 7:e45387
106. Hirasawa H, Betensky RA, Raviola E (2012) Corelease of dopamine and GABA by a retinal dopaminergic neuron. J Neurosci 32:13281–13291
107. Zhang D-Q, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG (2008) Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. Proc Natl Acad Sci U S A 105:14181–14186
108. Zhang D, Belenky MA, Sollars PJ, Pickard GE, Mcmahon DG (2012) Melanopsin mediates retrograde visual signaling in the retina. PLoS ONE 7:e42647
109. Barnard AR, Hattar S, Hankins MW, Lucas RJ (2006) Melanopsin regulates visual processing in the mouse retina. Curr Biol 16:389–395
110. Joo HR, Peterson BB, Dacey DM, Hattar S, Chen S-K (2013) Recurrent axon collaterals of intrinsically photosensitive retinal ganglion cells. Vis Neurosci 4:175–182
111. Johnson J, Wu V, Donovan M, Majumdar S, Rentería RC, Porco T et al (2010) Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A 107:17374–17378
112. Tian N, Copenhagen DR (2003) Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. Neuron 39:85–96
113. Renna JM, Weng S, Berson DM (2011) Light acts through melanopsin to alter retinal waves and segregation of retinogeniculate afferents. Nat Neurosci 14:827–829
114. Rao S, Chun C, Fan J, Kofron JM, Yang MB, Hegde RS et al (2013) A direct and melanopsin-dependent fetal light response regulates mouse eye development. Nature 494:243–246
115. Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L et al (2011) Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature 479:223–237
116. Davies WL, Hankins MW, Foster RG (2010) Vertebrate ancient opsin and melanopsin: divergent irradiance detectors. Photochem Photobiol Sci 9:1444–1457
117. Murakami M, Kouyama T (2008) Crystal structure of squid rhodopsin. Nature 453:363–367

Index

A
Adenosine, 159, 162
Gs-coupled, 157
Amacrine cell, 88, 107, 112, 125, 134, 145, 159
AII, 111, 112, 126, 127, 145, 146, 148, 149, 150–152, 154, 160, 187
dopaminergic, 122, 124, 125, 129, 188, 189

B
Bipolar cell, 17, 68, 87, 145, 150, 152, 187
ON, 3, 4, 83–85, 87, 88
rod, 50, 58, 82, 84, 85, 90, 124, 129, 130, 133–136, 160

C
Calcineurin, 108, 109
cGMP-dependent kinase, 111
Chromophore, 10, 24, 25, 28, 30, 178, 179
vitamin A, 178
Circadian, 4, 154, 189
photoentrainment, 189, 190
Cone-driven vision, 126
Congenital stationary night blindness (CSNB), 68, 74, 75, 102

D
Dark adaptation, 12, 27, 28, 30
prolonged, 148, 149, 152, 154, 159
Dopamine, 3, 4, 121, 126–129, 134–136, 147–149, 154, 161, 162, 188
in retina, 122, 124, 125
receptors, 130, 148, 150, 157, 159

E
Effector, 3, 4, 50, 87
G-protein, 54
Electrical synapse, 127, 128, 144, 145, 150, 151, 152, 159, 160
physiological functions of, 146

G
Ganglion cell, 4, 88, 93, 107, 111, 125–129, 145, 150, 152, 159, 186, 187
Gap junction, 4, 122, 126–128, 144–148, 150–152, 154, 159, 160
GPR179, 68, 69, 71, 74, 75, 91, 94
G protein, 1–4, 50, 101, 102, 104, 122, 178, 182, 183, 185
G Protein-Coupled Receptor Kinase (GRK), 13, 185
G protein-coupled receptors (GPCRs), 1–4, 24, 34, 68, 74, 121, 173, 174
G protein-coupled receptors (GPs), 68
Gαo, 69, 82, 84, 86, 88, 90, 92
Gβ3, 69, 83–87
Gβ5, 71, 74–76, 83, 88, 90, 91, 94
Gβγ, 2, 31–34, 103, 104
Gγ13, 84, 86, 87

H
Heterotrimeric G-proteins, 31
Horizontal cell, 85, 100, 125, 126, 128, 134, 135, 145, 147–149, 152, 154

I
Intrinsically photosensitive retinal ganglion cells (ipRGCs), 4, 17, 124, 174, 177, 179, 180, 182, 183, 185, 186, 188

© Springer Science+Business Media New York 2014

K. A. Martemyanov, A. P. Sampath (eds.), *G Protein Signaling Mechanisms in the Retina,* Springer Series in Vision Research 3, DOI 10.1007/978-1-4939-1218-6

centrifugal signaling from, 188–190
phototransducton in, 180, 181
synaptic input to, 186, 187

L
Ligand-opsin interactions, 25
Light adaptation, 14, 16, 27, 50, 122, 144, 147, 154, 160

M
Melanopsin, 174, 176–178, 180, 181, 183, 186, 189
cascade, 4
structural characteristics, 177, 178
Metabotropic glutamate receptor type 6 (mGluR6), 3, 4, 68, 69, 71, 74, 75, 82, 84, 85, 88, 89, 91, 94, 102, 103, 107, 112

N
Non-image forming vision, 179, 180, 190

O
Opsin, 25, 28, 30, 178

P
Phosphodiesterase 6 (PDE6), 49–53, 56, 57
Photopigment, 25, 178, 180, 182
Photoreceptor, 2, 14, 15, 25, 27, 28, 32, 34, 49, 58, 101, 104, 110, 125, 146, 160, 161, 177
gultamate, 68
Phototransduction, 3, 4, 14–16, 24, 35, 49, 50, 88, 180
cascade, 181–185
in rods and cones, 30–33
mechanisms, 9–12
regulation and modulation of, 185, 186
Purkinje cell protein-2 (PCP2), 92

R
Rate-limiting step, 14, 15
Recoverin, 11, 15, 16
Regulators of G-protein Signaling (RGS), 12, 13, 50, 88
RGS7, 71, 74–76, 88–92
RGS11, 71, 74, 75, 85, 88–91
Retina, 2–4, 14, 23, 28, 35, 82, 85, 100, 107, 124, 127, 128, 136, 144–146, 150, 180, 186, 189, 190
Rhodopsin, 2, 10–16, 31, 34, 57, 177
Rod and cone photoreceptors, 2, 4, 23, 26, 39, 84, 154
Rod-driven circuitry, 124, 130, 135
Rod-driven vision, 129
Rods, 11, 13, 14, 25–29, 33, 100, 101, 103, 126, 128, 173, 178, 180, 182, 188, 190
mouse, 16, 30, 32, 34, 35
salamander, 14
skate, 27

S
Signal transduction, 2, 4, 34, 182
Sign-inverting synapse, 114
Synaptic gain, 114

T
Transducin assembly, 34
Transient receptor potential melastatin 1 (TRPM1), 68, 69, 71, 74, 75, 77, 85, 87, 102–105, 108, 109, 112
Trp channel, 108, 182, 183

V
Visual cycle, 28, 179
Visual pigments, 4, 12, 14, 17, 28, 30