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Jan Kremers
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Human Color Vision

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About the Series

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 behaviour 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, postdoctoral 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. This volume on Human Colour Vision covers many recent developments in the field and provides descriptions of new methods and emerging hypotheses. Although relatively colour blind, or at least compromised, compared to some other animals, humans are particularly concerned with colour. We fill our world with it and in common with other species use colour for object detection and discrimination of certain features, basing many of our day-to-day judgements on colour differences. We therefore hope that this book will be of interest to anyone with interests in the biology of colour vision, the medical aspects of what happens when it fails, other areas of colour science and within the world of art and design.

Jan Kremers • Rigmor C. Baraas
N. Justin Marshall
Editors

Human Color Vision

Springer

Editors
Jan Kremers
University of Erlangen-Nürnberg
Erlangen, Germany

N. Justin Marshall
Queensland Brain Institute
The University of Queensland
Brisbane, Australia

Rigmor C. Baraas
Department of Optometry and Visual Science
University College of Southeast Norway
Kongsberg, Norway

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Preface

Color vision is considered to be a visual sensation that is intimately related to emotions. If the world is "colorful," then it is positively full of wonder and surprises. When it is "bleak" or "gray," then the prospects are sad and pessimistic. Red is warm but can mean danger. Blue is cold. Yellow is a color of warning.

Color vision is a marvelous subdiscipline in vision research, embraced by those who study it and sometimes carefully avoided by those for whom it is only of indirect interest.

In the recent years, our understanding of human color vision has been tremendously advanced by many new developments, ideas, and achievements. It has been and still is an exciting time for color vision scientists. We therefore think that it is timely that these new developments are brought together in a book, particularly if it is one in a series on Vision Research. In this book, many new developments have been assembled, covering many different levels from genetics to perception, and studied with state-of-the-art methods such as genetics, morphology, imaging techniques, electrophysiology, psychophysics, and computational neuroscience. The genetics of cone photopigments is discussed in Chap. 1. Further new exiting developments have been obtained in the study of cone mosaics (Chap. 3), in the physiology of color vision in retinal (Chaps. 2 and 4) and cortical circuitries (Chap. 7), and in color psychophysics and perception (Chaps. 5, 6 and 8). Going beyond the questions about the processes leading to visual perception within an individual, the book also considers the latest computational models (Chap. 9), clinical implications and the question how retinal disorders can compromise color vision (Chap. 10), and finally the evolution of color vision (Chap. 11). We hope that the reader will find the chapters inspiring and helpful in defining scientific topics that will be of interest in the future. We think that there will be many interesting challenges. To name but a few, the following topics may emerge: the molecular basis of color vision; the study of single cells and pathways and their visual responses in the living retina; the responses of cells in their intact circuitries; the mathematical description of color processing; the improved use of color vision in diagnosing and monitoring inherited and acquired disorders of the retina; a better understanding of the many perceptual aspects of color vision.

We were supported by world experts who contributed to the book and wrote chapters on the new developments in their field of interest. We encouraged them to seek contact and collaborate with other experts. The result often was an interesting discussion amongst the authors and with the editors. We are extremely glad and proud that all authors have put so much effort in writing their chapters. We asked the authors to keep the text as simple and understandable as they could (without compromising on the scientific content), so that it also would entice and interest nonexpert scientists and students. The result is a book of which we think highly of and we are confident that it brings the latest developments in color vision research for a broader scientific audience. We hope you, as a reader, will agree.

We would like to thank the authors for their brilliant efforts. We appreciate it enormously. The collaboration between the series editors and with Springer was also extremely positive and inspiring.

It remains to thank those who continuously supported us. More particularly:

Rigmor C. Baraas: Finn Erik, Rasmus, the rest of the family, and all present and former members of the lab.

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Justin Marshall: Sue for endless support and patience

Erlangen, Germany — Jan Kremers
Kongsberg, Norway — Rigmor C. Baraas
Brisbane, Australia — N. Justin Marshall

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Contributors

Rigmor C. Baraas National Centre for Optics, Vision and Eye Care, Department of Optometry and Visual Science, Faculty of Health Sciences, University College of Southeast Norway (USN), Kongsberg, Norway

John Barbur Applied Vision Research Centre, School of Health Sciences, City University London, London, UK

Livia S. Carvalho Lions Eye Institute, University of Western Australia, Perth, WA, Australia

Vebjørn Ekroll Laboratory of Experimental Psychology, University of Leuven (KU Leuven), Leuven, Belgium

Wolf M. Harmening Department of Ophthalmology, University of Bonn, Bonn, Germany

Cord Huchzermeyer Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany

David M. Hunt, Ph.D., F.R.S.B. Lions Eye Institute, University of Western Australia, WA, Australia

School of Animal Biology, University of Western Australia, Stirling Highway, Perth, WA, Australia

Gerald H. Jacobs Department of Psychological and Brain Sciences, University of California, Santa Barbara, CA, USA

Elizabeth N. Johnson Department of Neurobiology and the Duke Institute for Brain Sciences, Duke University School of Medicine and Duke University, Durham, NC, USA

Almut Kelber Department of Biology, Lund University, Lund, Sweden

Frederick A.A. Kingdom Department of Ophthalmology, McGill Vision Research, McGill University, Montreal, QC, Canada

Jan Kremers Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany

School of Optometry and Vision Science, University of Bradford, Bradford, West Yorkshire, UK

Barry B. Lee School of Optometry, State University of New York, New York, NY, USA

Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany

Declan J. McKeefry School of Optometry and Vision Science, University of Bradford, Bradford, West Yorkshire, UK

Kathy T. Mullen Department of Ophthalmology, McGill Vision Research, Montreal, QC, Canada

Maria Olkkonen Department of Psychology, Science Laboratories, Durham University, Durham, UK

Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland

Neil R.A. Parry School of Optometry and Vision Science, University of Bradford, Bradford, West Yorkshire, UK

Centre for Hearing and Vision Research, Institute of Human Development, University of Manchester, Manchester, UK

Vision Science Centre, Manchester Royal Eye Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK

Austin Roorda School of Optometry, University of California, Berkeley, CA, USA

Ramkumar Sabesan School of Optometry, University of California, Berkeley, CA, USA

Lawrence C. Sincich Department of Optometry and Vision Science, University of Alabama at Birmingham, Birmingham, AL, USA

Luiz Carlos L. Silveira Tropical Medicine Nucleus and Biological Sciences Institute, Federal University of Pará, Belém, Pará, Brazil

Ceuma University, São Luís, Maranhão, Brazil

William S. Tuten School of Optometry, University of California, Berkeley, CA, USA

Thomas Wachtler Computational Neuroscience, Department Biologie II, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

Christian Wehrhahn Vision Center Laboratory, The Salk Institute, La Jolla, CA, USA

Andrew J. Zele School of Optometry and Vision Science and Institute of Health and Biomedical Innovation, Queensland University of Technology (QUT), Brisbane, QLD, Australia

Chapter 1
The Genetics of Color Vision and Congenital Color Deficiencies

David M. Hunt and Livia S. Carvalho

Abstract Primates are unique among mammals in possessing trichromacy. In Old World primates, it is based on three cone classes in the retina, each expressing a different class of visual pigment. These pigment classes are each orthologues of pigments present throughout the vertebrate kingdom, the short wavelength-sensitive (SWS1, SWS2, LWS and MWS) pigment and two representatives of the long wavelength-sensitive (LWS) pigment, L cone opsin and M cone opsin. The latter two pigments arose from a duplication of the *LWS* gene that occurred at the base of the Old World primate lineage to give an array of two closely adjacent opsin genes on the X chromosome. This close proximity and the extensive sequence identity of the *L* and *M* genes promotes mispairing of the genes and thereby underlies the high frequency of red-green color blindness seen in humans. The consequences of this mispairing are the loss of either the *L* or *M* gene to give full dichromacy, or the generation of hybrid genes to give anomalous trichromacy. Generally, red-green color blindness is not associated with loss of acuity, although this is present in a rare form of dichromacy called Bornholm eye disease where cone dysfunction and myopia is also present. Other forms of color blindness include the X-linked disorder of blue cone monochromatism where L and M cones are absent, the dominant disorder of tritanopia where S cone are severely reduced or absent, and the recessive disorder of achromatopsia where all cone classes may be absent.

Keywords Color vision • Evolution • Color blindness • Trichromacy • Dichromacy • Achromatopsia • Visual pigments • Visual opsins

D.M. Hunt, B.Sc., Ph.D., FRSB (✉)
Lions Eye Institute, University of Western Australia,
Verdun Street, Perth, WA 6009, Australia

School of Animal Biology, University of Western Australia,
Stirling Highway, Perth, WA 6009, Australia
e-mail: david.hunt@uwa.edu.au

L.S. Carvalho, B.Sc., M.Sc., PhD
Lions Eye Institute, University of Western Australia,
Verdun Street, Perth, WA 6009, Australia
e-mail: liviacarvalho@lei.org.au

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1.1 Introduction

The vertebrate retina contains photoreceptors cells that are specialized for the capture of light. These cells are subdivided into two classes, the rods and cones. Rods are responsible for monochromatic vision in dim light and cones for vision at normal light levels and for color vision. In rod and cone photoreceptors, the outer segments are composed of a stack of membranous disks in which the key molecules for photon capture, the photosensitive visual pigments, are embedded.

The mechanism of color vision depends critically on a comparison of the amount of light (photon capture) falling on spectrally different types of cone photoreceptors that are maximally sensitive to different wavelengths (For further details see Chap. 4). This is the process of cone opponency whereby each photoreceptor type is stimulated to a different extent by light of differing spectral content. Comparison of these signals by the brain provides the sensation of color. From this it follows that color vision requires a minimum of two spectrally different types of cone photoreceptors to be present.

In primates, trichromatic color vision is provided by the presence of three classes of cone photoreceptors with wavelengths of maximal sensitivity (λ_{max}) in the yellow-green (around 560 nm, longwave-sensitive, L), green (around 530 nm, middlewave-sensitive, M), and blue (around 430 nm, shortwave-sensitive, S) regions of the spectrum [1–3]. The light-sensitive components of photoreceptors, the visual pigments, are members of the GPCR family of proteins. They comprise a seven transmembrane (TM) opsin protein that is covalently linked to a Lys residue via a Schiff base (SB) to the chromophore. In mammals, this is invariably 11-*cis*-retinal derived from vitamin A1, so the peak spectral absorption (λ_{max}) of a visual pigment is determined not by the chromophore, but by the amino acid sequence of the opsin protein, with certain residues tuning the pigment to particular spectral locations.

1.2 Phototransduction Cascade

The ability to see in dim and bright light arises in vertebrates from differences in the light sensitivity of rods and cones. In general, rod photoreceptors are more sensitive than cones, have less "dark noise', adapt over a much narrower range of light intensities, but have slower response kinetics [4–7]. In contrast, cones have a higher frequency of spontaneous thermal isomerizations of the chromophore [8], and a substantial proportion of cone opsin exists in an apo form lacking bound chromophore. This apo-pigment is able to activate phototransduction and may account, in part, for the faster and larger response of cones [9, 10]. The presence of either a rod or cone pigment however makes only a minor contribution to the kinetics of the photoresponse, as demonstrated by the photoreceptors present in the retina of the nocturnal Tokay gecko, *Gecko gecko*, which have a rod-like morphology [11] and rod-like photokinetics [12], but contain

only cone visual pigments [13]. The fundamental difference between rods and cones with respect to sensitivity to light is not therefore simply determined by the presence of either a rod or cone visual pigment.

The visual process involves the conversion of the signal from the light-activated visual pigment to an electrical impulse and this is achieved by the process of phototransduction within the photoreceptor; this process also results in a substantial amplification of the original signal. A striking feature of vertebrate photoreceptors is the number of rod- and cone-specific isoforms that form the phototransduction cascade (Fig. 1.1). Absorption of light causes the isomerisation of the chromophore, 11-*cis*-retinal, to the all-*trans* form in a photobleaching sequence with consequent conformational changes in the opsin protein, leading to the activation of the G protein transducin by the activated form of the visual pigment, metarhodopsin II (meta II) (Reviewed in Ref. [14]). Meta II activates the heterotrimeric GTP-binding protein transducin, which is composed of α, β, and γ subunits. GDP bound to transducin is replaced by GTP and the GTP-α-subunit conjugate dissociates from the βγ component. Different isoforms for all three of these subunits are present in rods and cones. Phosphodiesterase (PDE) in rods is composed of catalytic α and β-subunits and two inhibitory γ subunits, whereas the cone form is composed of two identical α′ subunits and two inhibitory γ subunits. Activation of PDE involves the interaction with GTP-α-transducin and the dissociation of the inhibitory γ subunits. This activation results in the breakdown of cGMP and the closing of the cGMP-gated (CNG) channels, which also differ between rod and cones, leading to reduced levels of intracellular Ca^{2+}. Cone channels are generally more permeable to Ca^{2+} than rod channels, and this may underlie, in part, the more rapid and larger light dependent changes in Ca^{2+} concentration in cone cells [15].

The restoration of the cGMP and Ca^{2+} resting states is achieved by the activation of retinal-specific guanylate cyclases (GCs) by the Ca^{2+}-binding guanylate cyclase activating proteins (GCAPs) [16]. At the low levels of Ca^{2+} that exist after the closure of the CNG channels, GCAPs activate the cyclase function of GC, resulting in the production of cGMP, the reopening of the CNG channels and an increase in Ca^{2+} concentration to the resting state level. Two isoforms of GC, GC1 and GC2, have been identified, but GC1 is clearly the more important enzyme for phototransduction since null mutations in the corresponding *GUCY2D* gene cause the severe blinding disease of Leber Congenital Amaurosis [17], and altered photoreceptor survival in gene knockout mutant mice [18]. Missense mutations are also a major cause of dominant cone dystrophy [19, 20] (see Chap. 10). *GUCY2D* is expressed in both rods and cones, although the level of expression is higher in the latter [21]. Multiple isoforms of retinal GCAPs have also been identified [22–25], but GCAP1 encoded by *GUCA1A* appears to play the more important role. Mutations in GCAP1 are known to cause dominant cone dystrophy [26–30].

The inactivation of the cascade is necessary for recovery from a photoresponse. This occurs as a two-step process, involving phosphorylation of the activated pigment (meta II) by rhodopsin kinase targeted to serine residues in the carboxy terminus of the opsin protein, followed by binding of the inhibitory protein arrestin [31]. Two retinal-specific kinases, GRK1 [32, 33] and GRK7 [34], are present in the

a

Gene	Subunit	Isoforms: Rod	Isoforms: Cone
Transducin	Alpha	*GNAT1*	*GNAT2*
	Beta	*GNB1*	*GNB3*
	Gamma	*GNGT1*	*GNGT2*
Phosphodiesterase	Catalytic	*PDE6A, PDE6B*	*PDE6C*
	Inhibitory	*PDE6G*	*PDE6H*
Cyclic nucleotide gated channel	Alpha	*CNGA1*	*CNGA3*
	Beta	*CNGB1*	*CNGB3*
Rhodopsin kinase		*GRK1*	*GRK7*
Arrestin		*SAG*	*ARR3*

b

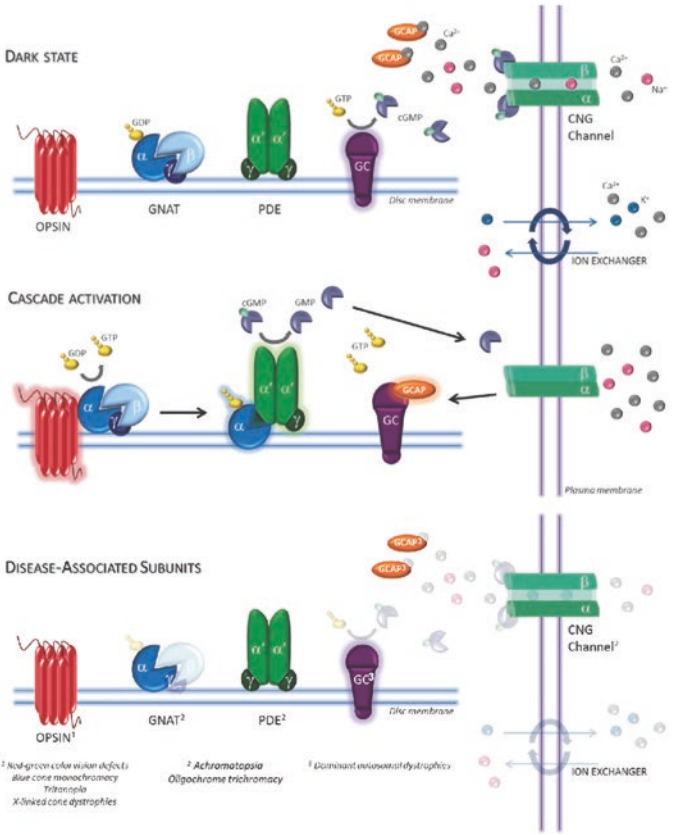

Fig. 1.1 Phototransduction cascade. (**a**) Gene isoforms of component processes that are expressed in either rods or cones. (**b**) Schematic diagram showing role of component processes in the activation of the cascade. D*ark state* shows the component processes with the cGMP-gated CNG channels open through the production of cGMP by activated retGC. *Cascade activation* results from the conversion of GDP to GTP and the release of the α-transducin subunit. This in turn activates PDE by removal of the inhibitory γ-subunit, leading to the breakdown of cGMP, the closure of the CNG channels and a fall in Ca^{2+} levels. Ca^{2+}-free GCAPs bind to retGC, enabling the activation of the cyclase activity and the production of cGMP. The restored levels of cGMP lead to a reopening of the CNG channels and the restoration of Ca^{2+} levels. *Disease-associated subunits* identify the component processes that are involved in defect in color vision

human retina; *GRK7* is preferentially expressed in human cones [35]. Inactivation is then completed by the binding of the inhibitory protein arrestin [31] to the phosphorylated terminus of the pigment; two isoforms of arrestin are present, S-antigen (SAG) in rods, and C- or X-arrestin (ARR3) in cones [36].

1.3 Evolution of Visual Pigments in Vertebrates

1.3.1 Ancestral Vertebrate Complement

The vertebrate blueprint for color vision consists of four spectrally distinct visual opsins, each encoded by a different gene, that first arose in the agnathans, as demonstrated by their retention by the southern hemisphere lamprey, *Geotria australis* [37, 38]. Orthologues of these four classes survived the split between the agnathans and gnathostomes which occurred over 540 MYA [million years ago] [39, 40] and have been retained by most vertebrate classes. They consist of a longwave-sensitive (LWS) pigment with λ_{max} 500–570 nm, a middlewave-sensitive (MWS or RH2) pigment with λ_{max} 480–530 nm, and two shortwave-sensitive pigment classes, SWS2 with λ_{max} 400–470 nm and SWS1 with λ_{max} 355–445 nm. In vertebrate visual pigments with λ_{max} values >385 nm, the SB is protonated, with a negatively charged residue at site 113 (usually Glu113) acting as a counterion to stabilize the proton of the SB [41]. In a subset of SWS1 pigments that show ultraviolet-sensitivity with λ_{max} values around 360 nm, the SB is unprotonated in the resting state [1, 42] (For an extended account of evolution of color vision see Chap. 11).

1.3.2 Loss of Cone Pigment Classes in the Early Evolution of Mammals

Not all of these cone pigment classes are found in mammals. Cone pigment loss is thought to have arisen during a nocturnal phase that marks the early evolution of the mammals around 150–200 million years ago (MYA) [43]. In marsupials and eutherian mammals, the *LWS* gene is paired with the *SWS1* gene, with the loss of the *SWS2* and *RH2* genes. The egg-laying protherian mammals, the platypus, *Ornithorhynchus anatinus*, and echidna, *Tachyglossus aculeatus*, belonging to the Order Monotremata that diverged from the marsupial/placental mammal lineage around 200 MYA, have also retained the *LWS* gene but this is paired the *SWS2* gene [44, 45]. In contrast therefore to marsupial and eutherian mammals, protherians have lost the *SWS1* and *RH2*. The presence of the *SWS2* gene in monotremes also means that ancestral mammals prior to the protherian–therian split must have retained both *SWS* genes which, in combination with the *LWS* gene, would have provided the basis for trichromacy.

1.4 Evolution of Trichromacy in Primates

1.4.1 Gene Duplication and Gene Conversion in Old World Primates

Amongst the mammals, only primates show true trichromatic color vision. The evolutionary drive behind the acquisition of trichromacy is thought to be improved color discrimination in the red/green region of the spectrum for the detection and evaluation of ripe fruits [46–49] and young nutritious leaves [50] against the green foliage of the rainforest. This has been achieved in Old World primates by a ~40 kb duplication of the X-linked *LWS* gene which occurred at the base of the Old World primate lineage [3, 51, 52] to give rise to an array of two adjacent genes (Fig. 1.2). The duplication generated a copy of the entire coding regions of the *LWS* opsin gene plus an almost complete copy of the *TEX28* gene [52, 53], a gene that is expressed in testis and not thought to be involved in vision. The duplicated *LWS* genes have subsequently diverged to give an upstream copy (*OPN1LW*) encoding a long wavelength-sensitive (L) pigment with λ_{max} around 560 nm and a downstream copy (*OPN1MW*) encoding a middle wavelength-sensitive (M) pigment with λ_{max} around 535 nm. In humans, intron 1 of the *L* opsin gene is generally longer by ~2.9 kb than intron 1 in the *M* opsin gene. This long intron is rarely present (1%) in the *M* opsin gene of Caucasians but is polymorphic in African-Americans at a frequency of 35% [54]. Individual cones express just one copy to give L and M cones, which together with S cones expressing the autosomal *SWS1* gene (*OPN1SW*), give rise to full trichromacy.

In females with two X chromosomes, the process of X chromosome inactivation will ensure that only one opsin gene array is active in any given photoreceptor, but another mechanism must exist that ensures that only one gene, either *L* or *M*, is switched on. This may be the role of the locus control region (LCR), a highly conserved segment of DNA located in humans between 3.1 and 3.7 kb upstream of the opsin gene array [55]. The LCR acts as an enhancer and is present in species where there is only a single X-linked opsin gene [52]; activation of this gene is nevertheless thought to require interaction between the LCR and the promoter region immediately

Fig. 1.2 Origin of opsin gene duplication on the X chromosome of Old World primates. The exons for the *L* and *M* opsin genes and for the *TEX28* gene are shown as *black bars. Arrows* indicate the direction of transcription. The positions of the upstream LCR and the minimal promoter for the *M* opsin gene are shown

upstream of the coding region [56]. Since the cone-specific phototransduction genes described above are all expressed in both L and M cones, the selection of either an *L* or *M* opsin gene for expression [57] would appear to be the single determinant for the production of either an L or M cone. This process may depend on the stochastic selection of one of two stable and mutually exclusive states. The *L* and *M* genes each possess a minimal promoter immediately upstream of exon 1, so this region could interact in a gene-specific manner with the LCR to activate only the adjacent gene. This was tested with transgenic mice that carried the human LCR and the L and M promoters driving different reporter genes within a transgene [58]. As shown in Fig. 1.3, the activity of the gene driven by the more proximal promoter to the LCR

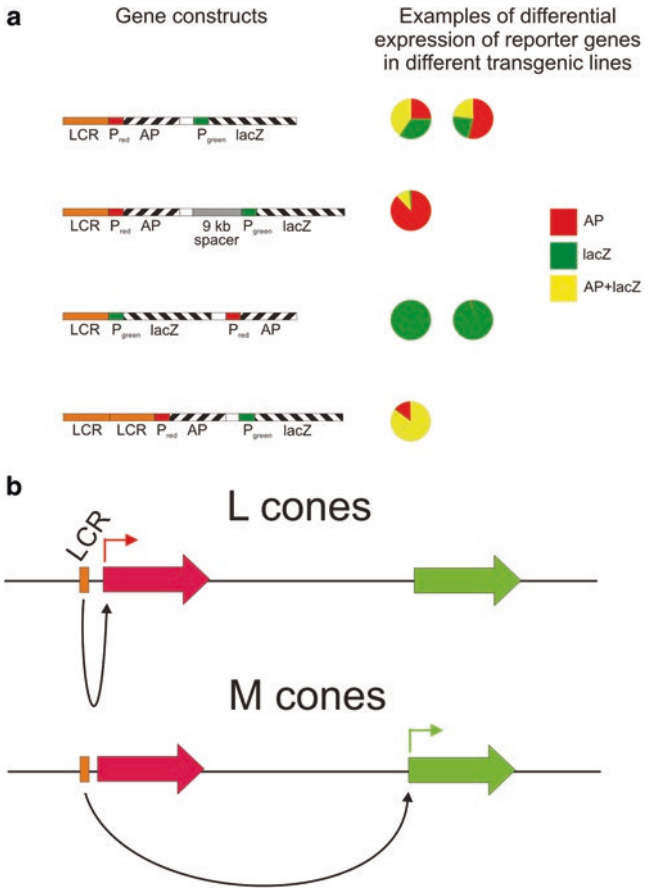

Fig. 1.3 The role of the LCR in directing reporter gene expression from *L* and *M* opsin gene promoters. (**a**) The relative activity of the *L* and *M* promoters in four different transgene arrays assessed in a number of transgenic mouse lines by scoring individual cone cells for the expression of the reporter genes. P_L human *L* opsin gene promoter, P_M human *M* opsin gene promoter, *AP* human placental alkaline phosphatase, *lacZ E. coli* β-galactosidase. Redrawn from Ref. [58]. (**b**) Potential mechanism for the stochastic expression of *L* or *M* opsin genes via LCR-opsin gene promoter interaction

was higher, while a 9 kb spacer interposed between the L and M reporter genes reduced the expression of the downstream gene even further, indicating that physical distance from the LCR may reduce the frequency of promoter activation. In this way therefore, only a single opsin gene within the array is activated, with the probability of interaction with either the L or M promoter dependent on the distance from the LCR and on certain key regions of the promoter, with different promoters showing stronger or weaker affinities [56]. This may also in part explain the differences in the relative number of L and M cones in different individuals (For more on L:M ratios see Chaps. 2, 3 and 5). Most subjects show a fourfold range [59] but this can extend in rare cases to a 30-fold range. It should be emphasized however that this variation in L and M cone ratios is not associated with color vision defects.

1.4.2 Spectral Tuning of Primate Visual Pigments

The spectral shifts between primate L and M pigments are largely due to substitutions at three sites, 180 encoded by exon 3 and 277 and 285 encoded by exon 5 [60], although smaller changes arise from substitution at 116 in exon 2, and 230 and 233 in exon 4 [61]. The residues present at the three former sites are polar Ser, Tyr, and Thr, respectively, in the L pigment and nonpolar Ala, Phe, and Ala, respectively, in the M pigment [51, 62]. As shown by site-directed mutagenesis and in vitro expression studies, the spectral shifts achieved by substitution at these sites are approximately additive [63, 64]. Site 180 is polymorphic in humans; Ser180 is the more common residue in the L pigment, but Ala180 is present at a significant frequency to give a short wavelength-shifted L pigment in some individuals [65].

UV-sensitive (UVS) SWS1 pigments are found throughout the vertebrate kingdom and almost certainly represent the ancestral form of the pigment [2, 66]. Within the mammalia, UVS pigments are relatively common in marsupials but are restricted in eutherians to just a subset of species from the Orders Rodentia, Chiroptera, and Insectivora [1]. All species of primates possess violet-sensitive (VS) pigments [67, 68] but the tuning method to change from UV to violet sensitivity remains uncertain. It has been proposed from a comparison with the mouse UVS pigment that tuning from UV to violet requires the simultaneous replacement of residues at three sites, Phe86Leu, Thr93Pro, and Ser118Thr [69, 70]. However, other residue changes at these sites are found in members of the Haplorrhini (New World and Old World monkeys and tarsiers), and the Strepsirrhini (prosimians excluding tarsiers), including Phe86 in one species, the aye-aye, a lemur endemic to Madagascar [71]. Phe86 is generally associated with a UVS pigment [66] and it is the replacement of this residue by either Tyr or Ser or Val [72–74] that is responsible for the loss of UV-sensitivity in a number of non-primate species [75]. In vitro expression of the aye-aye pigment however gave a peak sensitivity at 409 nm [71], so Phe86 does not in this case result in a UVS pigment. The only residue that is consistently present in primate VS pigments is Pro93. Pro93 is also found in the VS pigment of the clawed frog [76] so a Thr93Pro substitution may underlie primate VS pigments. Consistent with this is the observation that a Pro93Thr substi-

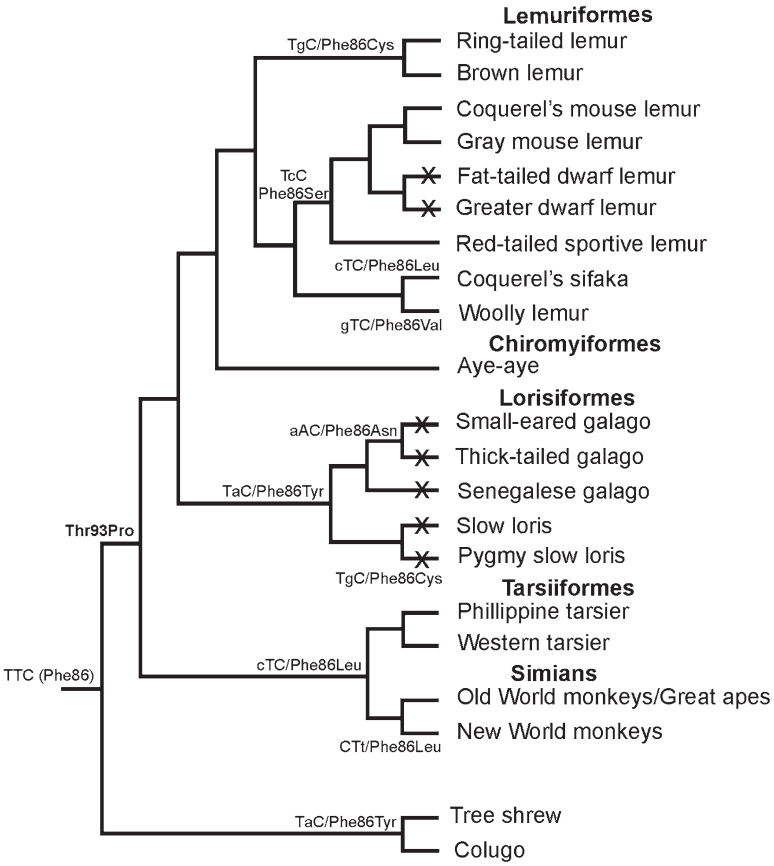

Fig. 1.4 Phylogenetic relationships of different primate species showing the different residues found at site 86 in S cone opsins, together with the corresponding codon sequences. Under the scenario presented, the Thr93Pro substitution occurred at the base of the primate lineage. Phe86 was present in the ancestral primate and retained by the aye-aye. Replacement of Phe by either Leu, Cys, Asn, Val, Ser, or Cys then occurred by single nucleotide changes at each step to generate the amino acid changes. Crosses on lineages indicate species where *SWS1* pseudogenes are present. Based on data from Ref. [78]

tution into the aye-aye pigment by site-directed mutagenesis causes a shift to 371 nm in the UV [71]. This does not however rule out a role for site 86 in the initial shift to violet sensitivity, especially since Tyr86 is found in the S cone opsins of the tree shrew and colugo [77], close relatives of the primates. As shown in Fig. 1.4, the most parsimonious scenario is that Phe86 was present at the base of the primate lineage but underwent a series of substitutions in the various primate lineages. A subset of these changes (Ser, Tyr, and Val) have been shown in other species to generate a shift into the violet [75], so these changes may have been responsible for the origin of the VS pigment in primates. However, if substitution at site 93 also occurred at the base of the primates, then this

Table 1.1 Prevalence of red-green color deficiency in European populations

Color vision deficiency	Cone(s) affected	Male prevalence (%)
Anomalous trichromacy		
Protanomaly	L	1.1
Deuteranomaly	M	4.6
Dichromacy		
Protanopia	L	1.0
Deuteranopia	M	1.3
Combined		8.0

Data from Ref. [80]

would have removed constraints on the residue at site 86, thereby allowing different substitutions at this site (which include Leu, Cys, Ser, Val, Tyr and Asn in different primate species) to fine tune the λ_{max} of the S pigment [71].

1.5 Color Vision Deficiencies and Color Blindness

1.5.1 L and M Opsins in Red-Green Color Vision Defects

The most common forms of color vision deficiencies in humans affect color discrimination in the red/green region of the spectrum and are associated with changes in the X-linked *L* and *M* opsin genes. Red-green color blindness is also referred to as Daltonism after John Dalton, the famous chemist, who was the first to describe the condition and who himself was red-green color blind [79]. The high frequency of these defects amongst males is a direct consequence of hemizygosity of X-linked genes (Table 1.1).

The original duplication in Old World primates generated a tandem opsin gene array on the X-chromosome, with the two copies diverging at key tuning sites to encode the spectrally distinct L and M opsin pigments. The coding regions of these genes share 98 % identity compared to only 40 % with S opsin, and the introns also show a high level of identity. A direct consequence of this close proximity and high level of sequence homology of the duplicate copies of the gene is mispairing during meiosis (Fig. 1.5). When this is followed by intergenic crossover within the array, extra downstream copies of the genes are placed into the array, with a consequent expansion of the number of copies of the *M* opsin gene. Copy number for *M* opsin is known to range from one to five with a mode of two [51, 54, 81, 82]. Mispairing within the array followed by intergenic crossover will also result in the deletion of gene copies and, where the crossover is intragenic between mispaired genes, this will give rise to hybrid genes. It is the latter two events that are responsible for the high frequency of red-green color vision deficiencies in humans [83, 84]. Dichromacy arises when either the *L* opsin gene (protanopia) or *M* opsin gene (deuteranopia) is missing; the prevalence among Caucasian males of these two forms of red-green color blindness is 1.01 % and 1.27 % respectively [80, 85]. Anomalous trichromacy arises from the production of hybrid *L*/*M* genes. The precise position of the intra-

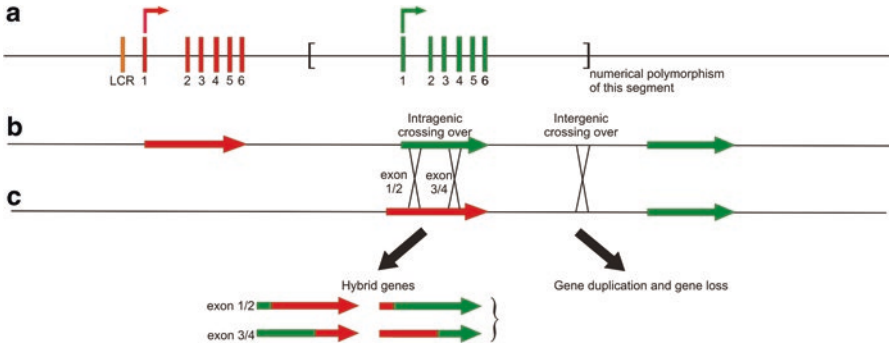

Fig. 1.5 Mispairing and crossover within the L and M opsin gene array. (**a**) Genomic organization of the L and M opsin genes. (**b**) Mispairing of the L and M genes, followed by either intragenic or intergenic crossover. (**c**) Gene duplication/loss and example products of intragenic and intergenic crossover between mispaired genes

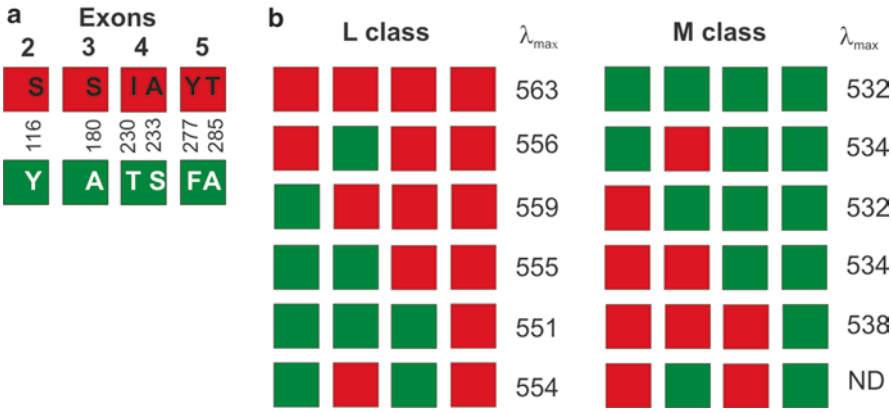

Fig. 1.6 Spectral tuning of L and M pigments. (**a**) Major spectral tuning sites in exons 2–5. (**b**) *L*, *M*, and hybrid genes and λ_{max} values for corresponding pigments. Data from Ref. [63]

genic crossover will determine the relative contribution of *L* or *M* sequence to the hybrid gene. Since the tuning sites 277 and 285 encoded by exon 5 make the major contribution to the spectral shift between L and M pigments, the location of the spectral peak will be largely determined by the origin of exon 5 from an *L* or *M* opsin gene. The most common form of anomalous trichromacy is deuteranomaly, where M cones express a hybrid pigment; the prevalence of this disorder in Caucasian males is 4.63 % [80, 85]. Protanomaly, where L cones express a hybrid pigment, is less common at 1.08 % in Caucasian males. With a few rare exceptions, only the first two genes in the array are expressed [86, 87] so L cone photoreceptors will express a normal L pigment whereas the M cone photoreceptors will express the hybrid pigment. Depending on the particular combination of *L* and *M* sequences in the hybrid gene (Fig. 1.6), there will be a reduction in the spectral separation between the two pigments, thereby reducing color discrimination in the red-green region of the spectrum.

The presence of multiple copies of genes within the opsin gene array and ongoing mispairing and nonhomologous crossover, has resulted in extensive intermixing of *L* and *M* opsin gene sequences. Only the first two genes in the array are generally expressed, so anomalous trichromacy will arise even when intact *L* and *M* opsin genes are present, if the first or second position in the array is occupied by a hybrid gene [84]. Protanomaly arises when a normal *L* opsin gene is absent, so the two pigments produced from the array come from a normal *M* and an *M*-like hybrid gene. It is however not possible to derive such a combination of genes from mispairing between arrays that have the ancestral organization of an upstream L and downstream M opsin genes, so such an arrangement must derive from multiple events [88]. This process of nonhomologous exchanges between genes and the generation of hybrids by mispairing and crossover has been referred to as gene conversion, a process whereby sequences of related genes become homogenized; in the present case, conversion has resulted in the coding and noncoding regions of the *L* and *M* opsin genes becoming more like each other within species than the same regions of either the *L* or *M* orthologues between species [62, 89–91]. In humans, it is only exons 2–5 that remain distinct, presumably due to natural selection acting on the tuning residues encoded by these exons [92]. Surprisingly, conversion is also seen in the X-linked opsin gene arrays of Old World monkeys, even though gene changes associated with red-green color vision deficiencies is extremely rare in nonhuman primates, with just two reports of genetic defects in the opsin gene array [93]. In the first study carried out in a colony of 744 male long-tailed macaques, *Macaca fascicularis*, three male animals were found to have a hybrid *L*/*M* gene [94], giving a frequency of anomalous trichromacy of 0.4 %, and in the second study involving 58 male chimpanzees, *Pan troglodytes*, an *L*/*M* hybrid was identified in just a single male [95]. Conversion is also seen in gibbons, but in this case, it is located in the introns of the *L* and *M* opsin genes [96]. Multiple *M* opsin genes is also seen in gibbons, with an average incidence of 23.5 % across all species studied. The corollary of this, that is the loss of genes from the array, was not seen, nor were any hybrid genes identified, in 152 individual animals genotyped. Overall therefore, this would appear to be strong evidence that red-green color vision deficiencies are highly detrimental to survival within nonhuman primate communities and are removed by natural selection.

1.5.2 Blue-Cone Monochromacy

Blue (S) cone monochromatism (BCM) is a rare X-linked congenital disorder that affects approximately 1 in 100,000 individuals. Affected males lack L and M cones so vision is dependent on S cones and rod photoreceptors alone [83, 97]. BCM typically presents with reduced visual acuity (6/24 to 6/60), pendular nystagmus and photophobia. Patients often have myopia, but the S cone ERG is well preserved [98].

The different genetic mechanisms underlying BCM all involve changes in the *L*/*M* opsin gene array, resulting in nonfunctional L and M photopigments and thus inactive L and M cones [63, 99]. These changes fall into three classes. The first class that accounts for approximately 60 % of cases involves a two-step process whereby nonhomologous recombination between the *L* and *M* opsin genes has reduced the number of genes in the opsin array to one, followed by a mutation in the remaining gene that inactivates the encoded pigment. Two missense mutations (Cys203Arg and Pro307Leu) and a nonsense mutation (Arg247Stop) have been reported [99–101]. Where a Cys203Arg mutation is the cause of BCM, it most commonly occurs in an *L*/*M* hybrid gene [102]. Imaging of the retina by adaptive optics in such individuals shows a relatively undisrupted cone mosaic even though cone density is reduced. This implies that cones expressing the mutant opsin undergo degeneration prior to final packing [103]. The second class of changes that accounts for approximately 40 % of cases involves a deletion upstream of the *L*/*M* opsin gene array that includes the LCR; the loss of the LCR eliminates expression from both the *L* and *M* opsin genes even though both may be fully intact and unaltered [101, 104–106]. Imaging of individuals with an LCR deletion showed that cone density was reduced and the organization of the mosaic was also disrupted [106]. Finally, exon deletion has been observed, either in an *L* opsin gene [107] or in a hybrid L/M gene [102]; in both cases, the array had been reduced to this single nonfunctional gene. BCM is generally considered to be a stationary disorder although longitudinal assessments have found some evidence for progression [100, 108].

1.5.3 Tritanopia

The loss of functional S cones, a condition called tritanopia, arises from mutations in the *S* opsin gene on chromosome 7. It is very rare compared to red-green color blindness with an estimated frequency of between 1 in 13,000 and 1 in 65,000 [109], although a later study puts the frequency much higher at 1 in 500 [110]. The absence of the blue-sensitive S pigment limits blue-yellow color discrimination. Tritanopia is inherited as an autosomal dominant disorder arising from missense mutations in the S opsin gene, which means that the presence of a single mutant copy of the S gene is sufficient to cause the visual defect. Reported amino acid substitutions caused by missense mutations are the replacement of a conserved Leu at site 56 with Pro (Leu56Pro) [111], Gly79Arg, Thr190Ile [112], Ser214Pro, Pro264Ser [113, 114], and Arg283Gln [115]. In all four cases, the substitutions are within the helical transmembrane domains of the opsin. Three of the substitutions involve either the gain or loss of Pro residues; since Pro residues introduce kinks, it would be expected to result in significant changes in the conformation of the pigment. The other changes involve either the gain (Gly79Arg) or loss (Arg283Gln) of a charged residue; in both cases, the change would be expected to impact adversely on pigment function. What is perhaps surprising is that the production of mutant opsin protein from a single mutant *S* opsin allele is sufficient to cause the loss of S

cone function, even though a normal allele encoding wild type S opsin is also present. In this regards, tritanopia is similar to the dominant forms of retinitis pigmentosa that arise from mutations in the rod opsin (*RH1*) gene [116–119]. Further evidence of the impact of these mutations comes from a study of two subjects (father and daughter) with the same Arg283Gln mutation but showing different degrees of color vision deficiency [115]. In vivo imaging shows that S cones are present at a near normal density in the daughter but totally absent in the father at an eccentricity in the retina where the peak S cone density normally occurs. It would appear from this family that tritanopia is a progressive disorder that shows incomplete penetrance in younger subjects.

1.5.4 Enhanced S-Cone Syndrome

Enhanced S-cone syndrome (ESCS), also known as Goldmann–Favre syndrome, is a recessive form of retinal degeneration in humans that is characterized by severely reduced rod sensitivity, loss of visual acuity, and atypical ERGs that show little or no responses to dim light (scotopic) stimuli, but have large, slow responses to brighter (photopic) stimuli [120–122]. This photopic ERG originates from an excess of S-cones in the retina, with reduced contributions from L and M cones. However, despite the reduction in L and M cone sensitivity, color vision in ESCS individuals is generally assessed to be normal. The genetic basis for ESCS has been shown by [123] to be due to mutations in the *NR2E3* gene. *NR2E3*, together with *NRL* [124], act as regulators of rod photoreceptor cell fate [125]. Both gene products are required for rod photoreceptor development, with *NR2E3* also required to suppress cone development [126]. In the absence of *NR2E3* expression, normal rod development is blocked [127], resulting in a severe reduction in rods and an increase in S cones.

The thyroid hormone system has been shown to play an important role in the development of cone photoreceptors. Thyroid hormone (TH) and the cone-specific TH receptor THRβ2, a nuclear transcription factor encoded by the *thrb* gene, have been shown to control the relative production of S and L opsin during cone development and maturation; in mice with a deletion for *thrb*, there is a selective loss of L cones with a concomitant increase in S cones [128]. In a recent study of an infant with a cone photoreceptor disorder associated with severe thyroid hormone resistance [129], a severely reduced photopic response was reported characterized by severely reduced L and M cone responses and an increased S cone response. The affected child was found to be a compound heterozygote for two missense mutations in the *THRβ2* gene, Arg338Trp and Arg429Trp. In both cases, the substitutions are in the ligand-binding domain, and it is suggested that the severity of the phenotype may be due to a selective disruption of homodimer formation, but not heterodimer formation, with retinoid X receptor (RXR) [130]. The increased S cone response in this disorder is similar therefore to ESCS. However, in ESCS, the increase in S cones is at the expense of rod photoreceptors whereas in this disorder, the enhanced S cone phenotype derives from a conversion of L and M cones to S cones.

1.5.5 Achromatopsia

Complete or incomplete achromatopsia (ACHM), also referred to as rod monochromacy, is characterized by reduced or complete absence of color vision caused by nonfunctional cone photoreceptors. It has been estimated to affect 1 in 30,000–50,000 people worldwide and has an autosomal recessive inheritance pattern [131, 132]. So far, mutations in five genes have been shown to cause achromatopsia, accounting for 93 % of patients [131, 133–139]. In all cases, the genes encode components of the phototransduction cascade (Fig. 1.1), with disease-causing mutations reported in genes encoding the α-subunit (*CNGA3*) and β-subunit (*CNGB3*) of the CNG channel, the α-subunit (*PDE6C*) and inhibitory γ-subunit (*PDE6H*) of cone PDE6, and the α-subunit of cone transducin (*GNAT2*). The mutational spectrum reported so far includes missense and nonsense, deletions and insertions, and splice mutations. However the vast majority of ACHM cases are due to mutations in the *CNGA3* and *CNGB3* genes, with *CNGB3* defects accounting for 50 % of total cases in the Caucasian population [140–142]. Of the remaining cases, mutations in *GNAT2* [137, 138, 143–146] and *PDE6C* [139, 147, 148] each have a prevalence of <2 %, while *PDE6H* disease variants have a prevalence of 0.3 % [136, 149].

Despite the small percentage of *GNAT2* and *PDE6C* mutation-related ACHM cases, the number of disease-causing variants reported for these genes has not been negligible, with 11 mutant variants reported for *GNAT2* [137, 138, 143–146] and 20 for *PDE6C* [139, 147, 148, 150, 151]. Interestingly, the majority of *GNAT2* mutations are nonsense changes while *PDE6C* has a much higher incidence of missense changes. Since both these α-subunits are highly conserved proteins that are integral for the correct activation of different components of the phototransduction cascade, the extremely low number of missense mutations (1/11) [137] in the *GNAT2* gene is surprising. Different subunit interactions between cone transducin and cone phosphodiesterase could account for different selective pressures and mutational tolerance between residues amongst these two genes.

The majority of ACHM cases are due to defects in the *CNGB3* gene, with the most common mutation being a 1 base pair frameshift deletion c1148delC (p.Thr383Ile fs*13); this accounts for around 70 % of *CNGB3* disease-causing alleles [140, 142, 152, 153]. Besides this frameshift deletion, more than 40 different disease-causing variants have so far been reported, with a high proportion of nonsense mutations [135, 142, 152, 154–158]. In contrast, disease-causing mutations in the *CNGA3* gene show greater variability with more than 80 variants described so far and a majority of missense mutations [153]. The CNG channel is a heterotetrameric complex of the α- and β-subunits [159] and this divergence in the type of variants found between the *CNGA3* and *CNGB3* genes aligns well with the different primary and modulatory functions of the α- and β-subunits, respectively [160], indicating a lower tolerance for genetic variance in the *CNGA3* gene.

Most of the reported ACHM studies looking at *CNGA3* and *CNGB3* mutations in large cohorts have been with patients of European or American origin or descent [161], which may give a skewed prevalence of disease variants. Recently, a large study of 130 ACHM patients distributed across 49 different families within the

Israeli and Palestinian populations determined the prevalence and spectrum of *CNGA3* and *CNGB3* mutations [157]. In this study, the most common cause of ACHM in western populations (*CNGB3*, c.1148delC) was only found in 8 % of families in their cohort while in 84 % of families, the disease was due to mutations in the *CNGA3* gene. The high incidence of *CNGA3* disease-variants in this population can be explained by the presence of two founder mutations in a common ancestor of Arab-Muslims and Oriental Jews. Another interesting finding of this study was the increased prevalence of ACHM in this population; extrapolation from these data indicated that ACHM prevalence could be as high as 1 in 5000 within the local Arab Muslim community, making it the second highest ACHM prevalence after the 4–10 % incidence in the population on the Pingelap Island in the Pacific Ocean [132]. Other studies have reported a different prevalence of *CNGB3* and *CNGA3* mutations between Northern European and Middle Eastern descent populations [162–164] but more studies using large cohorts of ACHM patients from differing genetic/populational backgrounds are needed to gain a more complete understanding of ACHM prevalence and disease-causing variants.

Clinical symptoms of ACHM start at birth/early infancy and include congenital pendular nystagmus, poor visual acuity, severe photophobia/hemeralopia, and severe defects in the protan, deutan, and tritan color axes [131, 140]. Hypermetropic refractive errors are common and affected subjects can also present with small central scotoma, eccentric fixation, and paradoxical pupillary responses [131, 149, 151, 165]. Fundus appearance is often normal, but macula changes have been reported, ranging from subtle retinal pigment epithelium (RPE) changes to atrophy and foveal autofluorescence [166]. Clinical symptoms can be highly variable from patient to patient and most studies have been unable to find a correlation between phenotype and genotype and/or specific disease variants, although one study comparing *CNGB3* and *CNGA3* patients reported a slightly more affected macular appearance and reduced visual acuity in the *CNGA3* patients [140].

Analysis of electroretinogram (ERG) and psychophysical color testing responses has led to the division of the disease into complete and incomplete ACHM subtypes [140]. Patients with the complete subtype have no recordable ERG cone function and completely lack of color discrimination, while those with incomplete ACHM retain some residual cone function on ERG, varying degrees of color discrimination and higher visual acuity [139, 143, 145, 162, 167, 168]. None of these studies however has been able to correlate the complete and incomplete subtypes with genotype, age or mutation, so it is possible that differences between ERG parameters and apparatus, psychophysical color vision tests and other traditionally used measures of cone vision amongst the studies could account for the discrepancy in the subtypes. An alternative explanation for residual cone function in some patients is that it is mediated by rods which in ACMH patients show a reduced desensitization under light-adapted conditions [156]. Historically, rod function in ACHM patients has been described as normal, although several recent studies, including some longitudinal recordings, have reported a reduction and/or deterioration of rod-driven ERG [140, 146, 157, 168–170] and rod-derived dark adaptation [170]. Rod dysfunction has

been reported in some but not all patients [140, 157, 168] and does not seem to correlate directly with genotype, with mutations in *CNGA3*, *CNGB3*, and *GNAT2* implicated. Surprisingly, abnormal rod function has not been reported in ACHM patients with *PDE6C* or *PDE6H* defects but this could be due to the relatively low number of cases. What is still unclear is why and how rod function is affected by nonfunctional cones. Hypotheses include structural deficit due to loss of cones, shortening of rods inner and outer segments, secondary apoptosis induced by dying cones and anomalies in the rod signaling pathway.

ACHM is usually considered a stationary disease with congenital symptoms and vision assessments remaining stable throughout a patient's life. However several cross-sectional and longitudinal studies have reported disease progression over time, mainly in *CNGA3* and *CNGB3* patients [140, 141, 158, 166, 168, 171, 172] but also in *GNAT2* [143, 172] and *PDE6C* [173] patients. Some of these studies indicate that there might be a higher rate of deterioration within the first years of life which then becomes stationary in older patients [171, 173]. Interestingly, assessments of ACHM mouse models have shown an initial peak in cone photoreceptor cell death which subsequently stabilizes [174–176], but the *CNGB3* deficient dog model was reported to have a continuous, but slow loss of cones [177]. If ACHM is indeed a progressive disorder, this will have important implications for future clinical trial design as treatment during the early stages of the disease may provide the best outcome, as shown by the presence of an optimal window of treatment for gene therapy in the *CNGB3* mouse [178] and dog models [177]. However, two recent studies have questioned the effect of age in disease progression [172, 179]; Sundaram et al. [179] failed to find a correlation between cone structure loss, deterioration in best-corrected visual acuity (BCVA), contrast sensitivity, or reading acuity with advancing age but did find a significant decline in microperimetry-based retinal sensitivity with age. This discrepancy with previous studies has been attributed to the cross-sectional nature of this study, genetic diversity of patients and lack of standardization in cone structure measurements between studies [180, 181]. However, in a follow-up study, the same group came to a similar conclusion that phenotype did not correlate with age or genotype and although ACHM may be progressive, it is slow and subtle in most patients [172]. This is the largest and longest prospective longitudinal study of ACHM to date [172] and it remains to be seen whether similar findings will be reported in other patient cohorts. Initial studies were unable to determine whether viable cone cells are still present in the retina of ACHM patients but several recent studies have taken advantage of the advancing technology in in vivo imaging to address this issue. Spectral-domain optical coherence tomography (SD-OCT) and adaptive optics scanning light ophthalmoscopy (AOSLO) have demonstrated a highly variable range of photoreceptor integrity and structure between patients and that although cone structure is most likely disrupted, cones are still present [179, 182–188]. These studies have not been able to identify an altered cone structure and cone prevalence in *CNGA3* and *CNGB3* genotypes, but one study has indicated that better cone structure preservation might be present in the *GNAT2*-based disease [185].

1.5.6 Oligocone Trichromacy

Oligocone trichromacy (OT) is a rare cone dysfunction syndrome [189], which is characterized by reduced visual acuity, mild photophobia, normal fundi, and a reduced amplitude of the cone ERG. However, color vision is within normal limits. It has been proposed that these patients might have a reduced number of normal functioning cones, with each of the three cone types affected equally, thereby maintaining normal proportions and permitting trichromacy [190]. Recent studies have indicated that OT, at least in some instances, may be caused by mutations in the same genes that cause achromatopsia, namely *CNGA3*, *CNGB3*, and *PDE6C* [191, 192]. Some forms of OT may therefore represent a mild form of achromatopsia.

1.6 Cone and Cone–Rod Dystrophies

1.6.1 Dominant Autosomal Dystrophies

Altered color vision is associated with a number of cone (COD) and cone–rod dystrophies (CORD) (see also Chap. 10 for clinical features), most likely arising from the progressive loss of cones in these disorders. One of the best characterized forms of COD arises from dominant mutations in the *GUCA1A* gene which encodes the retinal guanylate cyclase activating protein (GCAP1) [193]. GCAPs bind calcium and act as activators of retinal guanylate cyclase (retGC) in the restoration of cGMP levels in photoreceptors after light activation (Fig. 1.1). Disease causing GCAP1 mutations are located in the EF-hands of the protein which alter its ability to bind calcium and thereby downregulate retGC activity [16]. Detailed clinical assessment of patients carrying a Tyr99Cys substitution in EF2 of the GCAP1 protein [29] has shown that the disorder presents as a mild photophobia with reduced central vision and defective color vision, with all three color axes affected. As the disorder progresses, all color vision may be lost. Confirmation that cones are progressively lost comes from two studies, firstly in a mouse line carrying a *GUCA1A* transgene with a Tyr99Cys mutation [194] and secondly in a gene-targeted mouse line carrying a knockin Glu155Gly mutation into EF4 which also causes COD [195, 196]. In both cases, cone photoreceptors are progressively lost.

Dominant mutations in the gene for retGC type 1 encoded by the autosomal *GUCY2D* gene account for a significant proportion of cases of CORD [19, 20], with the majority of disease-causing changes involving replacement of Arg at site 837. Site 837 is in a coiled-coil region of the protein that is important for GCAP binding [197]; as for the *GUCA1A* mutations described above, mutation in this region of the retGC1 protein results in constitutive activation of cyclase activity [198]. Clinical assessment of a patient with a *GUCY2D* mutation revealed the absence of color discrimination on a tritan axis, with a significant retention of red–green discrimination [199], implying that S cones may be preferentially lost in this disorder.