Annu. Rev. Genet. 1992. 26:403–24

MOLECULAR GENETICS OF HUMAN VISUAL PIGMENTS

Jeremy Nathans, Shannath L. Merbs, Ching-Hwa Sung, Charles J. Weitz, and Yanshu Wang
Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

KEY WORDS: color vision, color blindness, retina, retinal disease

CONTENTS

INTRODUCTION

Human vision is based upon four light-sensitive pigments: in dim light, it is mediated by rhodopsin, the visual pigment in rod photoreceptors, and at higher light levels, it is mediated by three pigments that reside in three classes of cone photoreceptors.[1] The absorption spectra of rhodopsin and the cone

[1]The three cone pigments and the photoreceptors within which they reside have historically been referred to as "blue", "green", and "red", to indicate those colors to which their wavelengths of maximal absorption approximately correspond.

0066-4197/92/1215-0403$02.00

pigments consist of broad bell-shaped curves that differ from one another principally by translation along the wavelength axis (Figure 1). Each visual pigment absorption spectrum—equivalent to a probability curve of photon capture as a function of wavelength—determines the action spectrum of the photoreceptor within which that pigment resides.

All visual pigments contain a chromophore, 11-*cis* retinal (or in some instances 11-*cis* dehydroretinal), bound via a protonated Schiff's base to a lysine residue of an integral membrane protein. Light activates a visual pigment by photoisomerizing the retinal chromophore from *cis* to *trans* about the 11–12 double bond, an event analogous to the replacement of an antagonist by an agonist within the binding pocket of a hormone receptor. The photoactivated visual pigment catalyzes the activation of a G-protein (transducin) that in turn activates a cGMP phosphodiesterase. The resulting decline in cytosolic free cGMP concentration closes plasma membrane cation channels that are gated directly by cGMP, thereby decreasing the inward current and hyperpolarizing the cell (reviewed in 55,84).

Each of the four human visual pigment apoproteins is encoded by a separate gene (60, 62). The nucleotide sequences of genomic and cDNA clones encoding the four human visual pigments reveal 40–45% amino acid identity between rhodopsin and each of the cone pigments, and between the blue pigment and the red or green pigments. By contrast, the red and green pigments are 96% identical at the amino acid level. Each visual pigment sequence shows

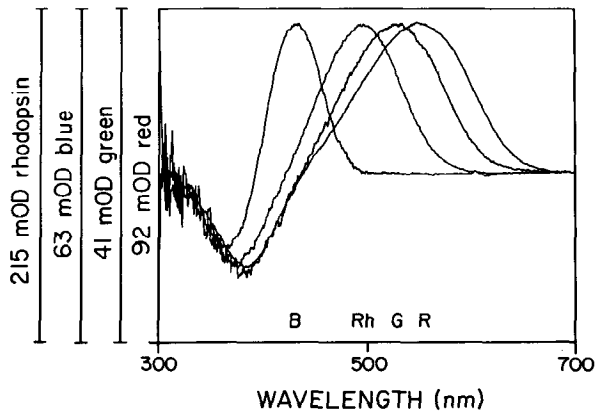

Figure 1 Absorption difference spectra of the four human visual pigments following in vitro reconstitution of the recombinant apoproteins with 11-*cis* retinal. Left to right: blue, rhodopsin, green, and red (Ala^{180}) pigment absorption spectra. Each photobleaching difference spectrum was obtained by subtracting an absorption spectrum measured after light exposure from one measured prior to light exposure. In the difference spectra, the positive peak is derived from the photolabile visual pigment, whereas the negative peak at 380 nm arises from released all-*trans* retinal, a product of photobleaching. (Adapted from ref. 57)

seven putative membrane-spanning segments, the most carboxy-terminal of which contains the conserved lysine to which retinal is attached.

In this review, we focus on sequence variation in the human visual pigment genes and the effects of this variation on visual physiology. Related aspects of human retinal physiology and pathology can be found in refs. (10, 35, 67, 73).

COLOR VISION

In 1860, James Clerk Maxwell described an instrument for producing and mixing monochromatic lights in defined proportions, thereby initiating the quantitative analysis of color vision (54). Maxwell showed that for most humans any stimulus light can be matched by a mixture of three spectrally pure lights of suitable wavelengths or by a mixture of two spectrally pure lights with the third added to the stimulus light. Human color vision is therefore said to be trichromatic.

The output from a single class of photoreceptors indicates the number of photoisomerizations per unit time for one type of visual pigment. From this output it is impossible to extract the independent variables of intensity and wavelength composition. Information regarding the wavelength composition of the stimulus, which we experience as color vision, requires a comparison between at least two classes of photoreceptors. In humans, color vision rests upon a comparison between the three classes of cones, which accounts for the three degrees of freedom that Maxwell observed in his color-matching experiments. Overlapping visual pigment absorption spectra are essential for this comparison so that a given stimulus produces a particular ratio of excitation of the three cone types (Figure 1). Rods appear to contribute little or no information to hue discrimination, principally because they are not active at high light intensities.

The three sensitivity curves upon which human color vision is based have been of long-standing interest to physiological psychologists (10). Maxwell showed that these sensitivity curves cannot be uniquely deduced from the color-matching data of normal trichromats; an infinite number of sets of three spectral sensitivity curves satisfy the trichromatic color-mixing equations (54). However, the three curves can be uniquely defined if one uses the additional constraints imposed by the color-matching data of subjects with dichromatic color vision (50,99; see below under Psychophysics of Red/Green Color Blindness). The curves obtained in this manner agree remarkably well with those determined by retinal reflectometry (79), microspectrophotometry (14), single cell-action spectra (82), electroretinography (66) and, most recently, by measurements of recombinant cone-pigment absorption spectra (57, 68). Photobleaching difference absorption spectra of the recombinant cone pigments have maxima at 426 nm for the blue pigment, 530 nm for the green

pigment, and 552 and 557 nm for two polymorphic variants of the red pigment (57).

RED AND GREEN PIGMENTS

Psychophysics of Red/Green Color Blindness

In analyzing his color-mixing experiments, Maxwell introduced a three-dimensional Cartesian coordinate system in which each axis represents the extent of excitation of one of the three receptors (54). Using this representation, Maxwell observed that the color space of individuals who are commonly referred to as color-blind occupies two rather than three dimensions. The axes that define the two-dimensional color space correspond to two of the three axes that define the usual three-dimensional color space. Maxwell correctly inferred that in these individuals one of the three receptor classes is nonfunctional. Their color vision is said to be dichromatic.

Maxwell studied the most common dichromacies, those in which either red or green receptors are nonfunctional. They are referred to as protanopia or deuteranopia, and abbreviated here as G^+R^- or G^-R^+, respectively. Affected individuals of either type have difficulty distinguishing stimuli at wavelengths greater than 500 nm because hue discrimination in this region of the spectrum relies only upon a comparison of the relative number of photons captured by the green and red pigments (Figure 1). Taking advantage of the low sensitivity of the blue receptors at long wavelengths, John William Strutt (better known as Lord Rayleigh) designed a simpler experimental paradigm to classify red/green anomalous subjects (76). In the Rayleigh test, the subject views a spectrally pure yellow light projected onto one half of a screen, and a superposition of red and green lights projected onto the other half. The subject adjusts the relative intensities of the red and green lights and the intensity of the yellow light until the two halves of the screen appear identical. In his original design, Rayleigh chose the sodium line at 589 nm for the yellow light, and the thallium and lithium lines at 535 nm and 670 nm for the green and red lights, respectively. Rayleigh observed that most trichromats reproducibly chose a particular red/green intensity ratio, and, as predicted, dichromats accepted any red/green ratio. Rayleigh also described a third class of subjects who require a red/green ratio that differs from the one chosen by the majority of trichromats. These subjects are referred to as anomalous trichromats. They can be divided into protanomalous or deuteranomalous trichromats, abbreviated here as G^+R' or $G'R^+$, depending upon whether the variation is in the red or green receptors, respectively. The Rayleigh match results suggest, and subsequent experiments have confirmed, that in these individuals the action spectrum of one of the receptors is shifted along the wavelength axis (70, 71, 81).

Both dichromacy and anomalous trichromacy arising from defects in the red or green receptors are inherited in an X-linked fashion. Among males, the frequency of all forms of variant red/green color vision is estimated to be 8% for European Caucasians, 4–5% for Asians, and 1–4% for Africans and American Indians (75). The frequency among females is less than the square of the male frequency, consistent with a two-locus model (31). Among colorblind European males the frequency of G^+R^- is 15%, G^-R^+ is 16%, G^+R' is 12%, and $G'R^+$ is 57% (31, 72).

Molecular Genetics of Red/Green Color Blindness

Molecular cloning of the red and green pigment genes has shown that the two are highly homologous and are arranged in a head-to-tail tandem array on the X-chromosome (25, 61, 62, 88). In individuals with normal color vision a single red pigment gene resides at the edge of the array with its 5′ end abutting unique flanking DNA. Typically between one and three, but in some cases as many as five, green pigment genes are found 3′ of the red pigment gene (20, 44, 62). Each repeat unit is comprised of 24 kb of intergenic DNA and a transcription unit of six exons occupying approximately 15 kb. The red and green pigment gene repeats are 98% identical at the DNA sequence level, including intron, exon, and 3′ flanking sequences; different green pigment gene repeats are 99.9% identical.

The proximity and high degree of sequence homology between repeat units apparently predisposes the array to unequal homologous recombination events. The location of the red pigment gene adjacent to unique flanking DNA leads to an asymmetry in red and green pigment gene rearrangements. Unlike the red pigment gene, each green pigment gene is flanked by 24 kb of nearly identical intergenic DNA, leading to duplication and deletion of green but not red pigment genes (Figure 2). Intra- and intergenic recombination events produce hybrid genes and changes in gene number, respectively. Based on an analysis of 93 males with variant red/green color vision, these types of recombination events appear to be responsible for > 97% of red/green variant genotypes (15, 19, 61, 65)

Intragenic recombination events between red and green pigment genes that replace the normal red pigment gene with a 5′red-3′green hybrid gene produce both G^+R^- and G^+R' phenotypes (Figure 2). The reciprocal recombination product, in which a normal green pigment gene is replaced by a 5′green-3′red hybrid, is usually associated with a $G'R^+$ phenotype if it occurs in an array containing additional green pigment genes. In the absence of additional green pigment genes the phenotype may be either $G'R^+$ or G^-R^+, depending presumably upon whether the hybrid gene encodes an anomalous or a red-like pigment, respectively. Theoretical considerations, and limited experimental data (15, 59, 65), suggest that only nine different hybrid pigment genes are present at appreciable frequencies in the human gene pool. These hybrids

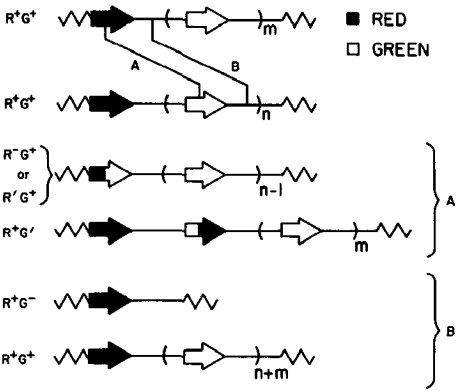

Figure 2 Unequal recombination within the tandem array of red and green pigment genes responsible for the common anomalies of red-green color vision. Each gene is represented by an arrow: the base corresponds to the 5′ end and the tip to the 3′ end. Filled arrows, red pigment genes; open arrows, green pigment genes. Unique flanking DNA is represented by zig-zag lines, and homologous intergenic DNA by straight lines. Two wild-type gene arrays, each containing one red pigment gene and a variable number of green pigment genes, are shown at the top. Intragenic and intergenic recombination events are indicated by A and B, respectively. For each recombination event, the reciprocal products are shown. Phenotypes: R^+G^+, normal trichromacy; $R'G^+$ red anomalous trichromacy (protanomaly); R^-G^+ red defective dichromacy (protanopia); R^+G' green anomalous trichromacy (deuteranomaly); R^+G^-, green defective dichromacy (deuteranopia).

arise from homologous recombination events between those exons that differ in sequence between red and green pigment genes, i.e. exons 2 and 3, 3 and 4, or 4 and 5. As a consequence of the uniformly distributed 98% nucleotide sequence identity between repeat units, and the 30- to 100-fold greater distance between the nearest sequence differences in adjacent exons compared to the distance between the farthest sequence differences within a single exon, most intragenic homologous recombination events fall outside of the sequence differences within a given exon, and, therefore, produce an *en bloc* shuffling of red or green pigment-specific exon differences. Nine rather than six hybrids are expected because the red pigment gene exists in two common allelic forms that differ by the presence of either Ala or Ser at position 180 in exon 3 (see below under Variation in Normal Color Vision).

Psychophysical experiments in the early 1970s demonstrated that the absorption maxima of the anomalous pigments in G^+R' and $G'R^+$ subjects lie between the normal red and green pigment absorption maxima (70, 71, 81). A recent study of the absorption properties of hybrid pigments produced from cloned cDNA shows that this pattern holds for each of the nine hybrid pigments with crossover points between exons (57a). These experiments show that for each hybrid pigment the position of the absorption spectrum along the wavelength axis depends both on the point of crossover and upon which of the two red pigment gene alleles contributed to the hybrid. Among 5′red-

3′green hybrid pigments in which position 180 in exon 3 is occupied by Ala the absorption spectrum approximates that of the green pigment (lambda max = 530 nm). If position 180 is occupied by Ser, a 3–4 nm red shift is observed relative to the parental green pigment. Additional red shifts of several nm are produced by red-specific amino acid sequences within exons 2–4. The absorption spectra of 5′green-3′red hybrid pigments in which exons 1 and 2 derive from the green pigment gene and exons 3–6 derive from the red pigment gene are affected by the Ala/Ser polymorphism at position 180 in a manner similar to that described above for 5′red-3′green hybrid pigments, absorbing maximally at 550 nm and 553 nm for alanine and serine versions, respectively. 5′green-3′red hybrid pigments in which the recombination event occurred between exons 3 and 4 or exons 4 and 5 absorb maximally at 549 nm or 545 nm, respectively, as compared to 552 nm or 557 nm for the two types of red pigment. Taken together, these data indicate that residues in exons 2, 4, and 5, as well as position 180 in exon 3 are important in determining absorption differences within this family of pigments.

The red and green pigments differ at 15 amino acids. Six of these differences involve conservative substitutions of hydrophobic residues. Of the remaining nine differences, seven involve the substitution of similarly sized residues that differ by the presence or absence of a hydroxyl group. Most likely, spectral tuning in this region of the spectrum involves differences in the intrinsic dipole moment of amino acid side chains carrying or lacking hydroxyl groups. Given that an absorption shift of 10 nm at a wavelength of 550 nm corresponds to a photoexcitation energy difference of only 1 kcal/mol, it is reasonable that dipoles of this strength could provide the requisite perturbation of the retinal chromophore (34). Predictions regarding the role of individual amino acid substitutions have recently come from correlations between primate visual pigment sequences and the absorption spectra of the corresponding pigments as determined by electroretinography (66) or microspectrophotometry (39, 95). Based upon a comparison of eight primate visual pigments, Neitz et al (66) proposed that red-shifts of 6, 9, and 15 nm are produced by substitution of Ser for Ala at position 180 in exon 3, Tyr for Phe at position 277 in exon 5, and Thr for Ala at position 285 in exon 5, respectively. A second set of comparisons have led to the proposal that substitutions at position 233 in exon 4 may also influence the absorption spectrum (39, 95). The data obtained from recombinant human hybrid pigments are in good agreement with these models of spectral tuning.

Variation in Normal Color Vision

Individuals who are considered to have normal color vision often show subtle differences in color-matching. An early description of this variability can be found in Maxwell's 1860 paper (54), in which he attributed the discrepancy in matches made by his subject "K" and himself to differences in the quantity and distribution of macular pigment, an inert yellow compound in the central

retina. Some individual variability can also be ascribed to a progressive yellowing of the lens with age. However, recent work has shown that in the long wavelength region of the spectrum, red pigment gene sequence polymorphism plays the most significant role in generating person-to-person variability in color-matching (57, 63, 96). Psychophysical experiments in the 1960s and 1970s demonstrated variability in red pigment absorption spectra among G^-R^+ dichromats, and in the absorption spectra of red and/or green pigments among normal trichromats (2, 4–6). Among male trichromats, Rayleigh matches are observed to cluster in a nearly symmetric bimodal distribution in which the separation between the two modes is consistent with a several nm variation in red pigment absorption spectra (63, 64, 96). Female trichromats show a trimodal distribution in which two modes, each representing approximately one quarter of the population, coincide with the male modes, while the third mode, representing one half of the population, lies in the interval between them (63). The segregation of Rayleigh matches within a family indicates that this variability is an X-linked trait and that the central mode is comprised of heterozygous females (89).

As noted earlier, there are two common alleles of the red pigment gene that differ by the presence of Ala or Ser at position 180. Two lines of evidence indicate that this allelic variation is responsible for the bimodal distribution of Rayleigh matches. First, in a sample of 50 males with normal color vision, 62% were found to have Ser and 38% were found to have Ala at position 180 in the red pigment gene (96). The distribution of Ala^{180} alleles correlates well with that mode in the distribution of Rayleigh matches that requires a more intense red light, and the distribution of Ser^{180} alleles correlates well with the mode that requires a less intense red light (Figure 3A). Second, recombinant human red pigments with Ala or Ser at position 180 absorb maximally at 552 or 557 nm, respectively (Figure 3B), in good agreement with both the genotype correlation and psychophysical predictions (57). A less common sequence polymorphism, in which Thr and Ser replace Ile and Ala at positions 230 and 233, respectively, in exon 4 of the red pigment gene, produces an independent requirement for more red light in the Rayleigh match (96), in agreement with the analyses of primate sequences described above (39, 95).

As a consequence of random X-inactivation, females who are heterozygous for an X-linked trait are somatic mosaics. Psychophysical experiments show that in females who are heterozygous for a red/green color vision defect, each retina contains a mosaic of normal and abnormal photoreceptors (12, 33). For example, heterozygotes make a large number of errors when asked to identify the colors of small stimuli that are presented too briefly to allow any significant eye movements. It seems possible, therefore, that females who are heterozygous for variant red or green pigments are functional tetrachromats. This conjecture is based upon an analogous situation in New World primates, in which female heterozygosity dramatically increases chromatic discrimination

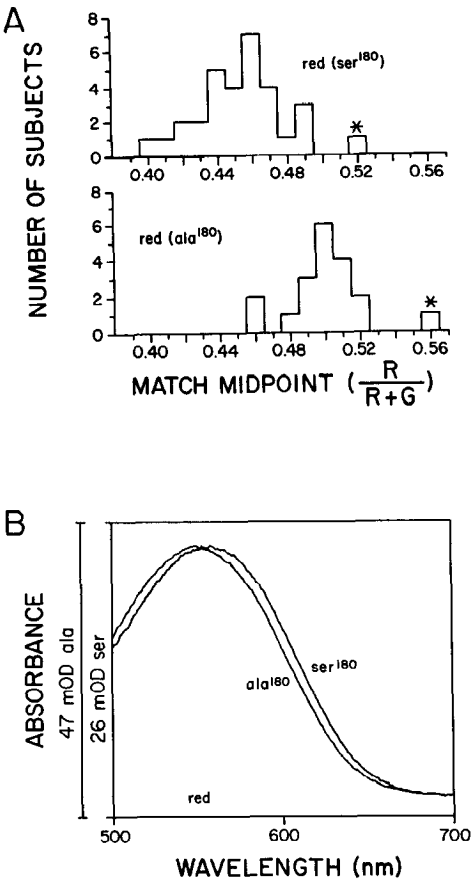

Figure 3 An Ala/Ser polymorphism at position 180 accounts for the bimodal distribution of Rayleigh matches among males with normal trichromatic color vision. (A) Histogram showing the number of individuals (vertical axis) with red pigment genes containing either Ser^{180} (upper) or Ala^{180} (lower) who choose a given ratio of red:red + green intensities to match a yellow standard (horizontal axis). Asterisks indicate individuals with red pigment genes containing Thr and Ser rather than Ile and Ala at positions 230 and 233, respectively. (Adapted from ref. 96.) (B) Absorption difference spectra of recombinant red pigments containing either Ser or Ala at position 180. (Adapted from ref. 57)

(42). Among squirrel monkeys, three alleles of a single X-chromosome-encoded cone pigment gene produce pigments with absorption maxima of 538, 551, and 561 nm. In behavioral tests of chromatic discrimination, male squirrel monkeys invariably test as dichromats, whereas heterozygous females test as trichromats. These observations suggest that there is sufficient neural plasticity within the primate visual system to accommodate an additional class

of cones. Among the 50% of human females who are heterozygous for the red pigment Ala/Ser polymorphism at position 180, any increase in discrimination is likely to be of low chromatic resolution because of the similarity in the two red-pigment absorption spectra.

To date no phenotypic effect has been ascribed to differences in the number of "extra" green pigment genes in the array. The relative efficiency of photon capture by the red and green cones, which shows a several-fold variation among individuals with normal color vision (80), does not correlate with green pigment gene number (T. P. Piantanida & J. Nathans, unpublished observations). Among African-Americans with multiple green pigment genes, 21% carry 5′green-3′red hybrid genes, far higher than would be expected based on the 4% frequency of all types of red-green variant color vision in this population (44). Moreover, in a survey of 129 color vision normal Caucasian males, four were found to carry a 5′green-3′red hybrid gene (15). These observations suggest that some genes in the array are either not expressed or are expressed at reduced levels. This hypothesis has recently been tested by PCR amplification of green pigment mRNA sequences from postmortem male retinas (J. Winderickx, L. Battisti, A. Motulsky & S. Deeb, personal communication). In each of 10 cases in which two green pigment genes could be distinguished, expression of only one was detected.

Blue Cone Monochromacy

True colorblindness, i.e. the absence of hue discrimination, is extremely rare, probably affecting not more than one person in 100,000. Two well characterized inherited varieties exist, rod monochromacy and blue cone monochromacy (1, 73). Rod monochromacy is an autosomal recessive trait in which apparently normal rods subserve all visual function. Blue cone monochromacy, also referred to as π_1, atypical, or incomplete achromatopsia, is an X-linked trait in which green and red cones do not function (G^-R^-). In dim light, the vision of blue cone monochromats is mediated by rods, and in bright light, it is mediated by blue cones (37). Interestingly, at intermediate light levels, blue cone monochromats show a weak interaction between rod and blue cone signals which permits crude hue discrimination (78).

In the normal retina, cones are most concentrated in the fovea, a small depression in the retina centered on the optical axis. The fovea subserves high acuity vision, with the highest acuity deriving from the central region of 100 microns in diameter. The central region contains only red and green cones, in contrast to the surrounding fovea, which contains all three cone types, a pattern that may have evolved to minimize the effects of chromatic aberration (90). Because blue cone monochromats lack functional red and green cones, they experience a profound decrease in visual acuity: the average acuity in adult blue cone monochromats is 20/200 (i.e. letters that would be legible to the normal observer at a distance of 200 feet are only legible when viewed

at a distance of 20 feet or less). In some blue cone monochromats, this deficit is made more severe by a progressive degeneration of the central retina (28, 59). The degeneration can be seen through the ophthalmoscope in some older blue cone monochromats; it is rarely seen prior to the third decade of life. A molecular analysis of the red and green pigment genes in 38 blue cone monochromat families has revealed two general classes of DNA rearrangement (Figure 4; 59; J. Nathans, unpublished observations). In one class, found in 20 families, the red and green pigment gene array is reduced by unequal homologous recombination to a single red or 5′red-3′green hybrid gene. In 16 of these families, the coding region of the red-green hybrid gene differs from those of the normal red and green pigment genes by a Cys-to-Arg mutation at codon 203 (Figure 5). Cys^{203} is the homologue of Cys^{187} in bovine rhodopsin, which forms one half of an essential disulfide bond (46, 47). Most likely, the Cys^{203}-to-Arg pigment fails to form a stable tertiary structure.

Recent experiments show that the Cys^{203}-to-Arg mutation is relatively common in the gene pool (97). One male with impaired green cone function was found to carry Cys^{203}-to-Arg substitutions in each of three green pigment genes. He represents the first clear exception to the pattern of red/green dichromacy or anomalous trichromacy arising from unequal homologous recombination events. This mutation was also found in 1 of 65 males with normal color vision. The presence of additional green pigment genes presumably accounts for the normal color vision of these two subjects.

The second general class of blue cone monochromat genotype, found in 14 families, consists of nonhomologous deletion of sequences upstream of and, in some instances, also within the red and green pigment gene cluster.

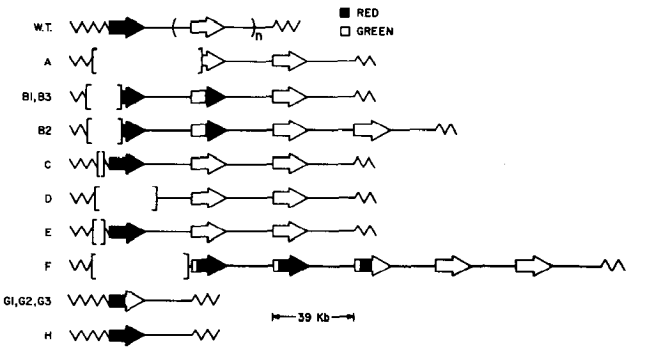

Figure 4 Mutations in and adjacent to the red and green pigment gene cluster responsible for blue cone monochromacy in 12 families. Symbols are defined in the legend of Figure 2. Each line indicates a different genotype, and each family is designated by a different letter/number combination. A wild-type gene array is shown at the top. Six different deletions are shown (indicated by brackets and designated by different letters); the two genotypes at the bottom of the figure carry a single gene as a result of homologous unequal recombination (From ref. 59).

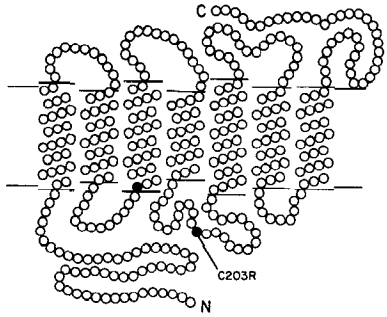

Figure 5 Schematic representation of a 5′green-3′red hybrid pigment showing the locations of Cys^{126} and Cys^{203} (filled circles). The seven alpha-helical segments are shown embedded within the membrane. The Cys^{203}-to-Arg mutation is designated by the single letter code for the wild-type amino acid, the codon number, and the introduced amino acid. N and C denote amino- and carboxy-termini, respectively. The amino-terminus faces the extracellular space.

The deletions range in size from 0.6 kb to 55 kb. Seven different deletions have been defined, and each is missing a 0.6 kb region that is absent from the chromosome with the smallest deletion (Figure 4c). A DNA rearrangement that includes part of the red pigment gene has also been reported in a family in which red cone function is missing, green cone function is greatly reduced, and a progressive degeneration of the central retina is observed (77).

Based upon the blue cone monochromat phenotype, it is reasonable to suppose that DNA within the 0.6-kb region acts as a long-range transcriptional control element (locus control region, LCR) that is required for the activity of all of the visual pigment genes in the red-green array. In support of this model, recent experiments indicate a requirement for these sequences in directing cone-specific expression of a reporter gene in transgenic mice (90a). Moreover, this small region contains sequences with a high degree of homology to sequences upstream of the mouse and bovine long-wavelength pigment genes (90a). The postulated affect of this flanking sequence on transcription units located over 40 kb away is reminiscent of the activity of the LCR located upstream of the beta-globin gene cluster, deletion of which results in beta-thalassemia (91). By analogy to models in which embryonic and adult chicken globin genes compete for an enhancer (11), the presence of a single LCR adjacent to the red and green pigment gene array suggests a mechanism by which each red or green cone expresses only a single type of visual pigment gene. If transcription of a given red or green pigment gene requires that the LCR physically interact with the promotor region, and if the LCR accommodates only one such interaction, then the formation of stable LCR-promotor complexes might be the critical event in determining red vs green cone identity.

THE BLUE PIGMENT

Psychophysics of Tritanopia

Tritanopia is a disorder of color vision characterized by poor chromatic discrimination in the short wavelength region of the spectrum and poor discrimination between colors that differ in the amount of blue admixed within them, e.g. white and yellow (73). In psychophysical tests, tritanopes perform as dichromats who lack the blue-sensitive mechanism. Although individual cases of tritanopia were reported a century ago (18, 53), congenital tritanopia was not recognized as a distinct inherited condition until Wright identified several dozen tritanopic subjects by publishing a color vision test in *Picture Post* (45, 98). Like the common forms of inherited variation in red-green discrimination, tritanopia occurs without signs or symptoms of generalized retinal disease. Unlike the red and green pathways, the blue pathway appears to be particularly susceptible to damage, which can lead to its acquired loss secondary to other ophthalmic disorders (73). For example, a disorder resembling tritanopia is typically seen as a secondary manifestation of dominant juvenile optic atrophy (52, 58).

Electroretinographic records obtained from tritanopes in three unrelated families show a diminished or absent response to stimuli selective for blue cones, indicating that the defect occurs within the retina, most likely at the photoreceptor level (58, 69). The possibility that some tritanopes have a partially functional blue-sensitive pathway is suggested by the observation that many affected subjects who perform as dichromats with stimuli subtending 1 degree of visual field, make quantitatively normal trichromatic color matches with stimuli subtending 8 degrees (74). Simultaneous activation of a larger number of blue cones may allow an impaired blue-sensitive pathway to contribute more effectively to color perception.

Tritanopia is unique among inherited disorders of color vision in its autosomal dominant transmission (45, 94). Earlier estimates of its prevalence in populations of European descent were 1 in 10,000 or less (45), but a recent study in the Netherlands, using testing methods with improved sensitivity and reliability, suggests that it may be as high as 1 in 500 (87).

Molecular Genetics of Tritanopia

Two groups have recently tested the hypothesis that mutations in the gene encoding the blue-sensitive pigment, located on chromosome 7 (61), can cause tritanopia. In one study, 7 of 9 unrelated tritanopic subjects were found to carry one of three different amino acid substitutions in the blue pigment gene: Gly^{79}-to-Arg in two Japanese subjects, Ser^{214}-to-Pro in two Caucasian subjects, and Pro^{264}-to-Ser in three Caucasian subjects (92, 93). In an independent study, the Pro^{264}-to-Ser mutation was found in the tritanopic members of two Caucasian families (T. Li, Zierath, P., Went, L. N., Smith,

V., Pokorny, J., Cho, N. J., Applebury, M., personal communication). The three mutations are absent from control subjects of matched ancestry, and coinherit with tritanopia in an autosomal dominant fashion, showing either incomplete penetrance (Gly^{79}-to-Arg), almost complete penetrance (Ser^{14}-to-Pro), or complete penetrance (Pro^{264}-to-Ser). Some of the apparent differences in penetrance may reflect differences in testing methodology.

The three amino acid substitutions responsible for tritanopia are located within putative alpha-helical membrane-spanning segments of the blue pigment, and each involves a significant change in chemical properties (Figure 6). It seems reasonable to suppose that they may perturb the folding and/or stability of the blue pigment, resembling in this regard many of the amino acid substitutions in rhodopsin that are responsible for one subtype of autosomal dominant retinitis pigmentosa (ADRP; see below under Inherited Defects in Rod Vision). In both tritanopia and ADRP the dominant nature of the disorders suggests that the mutant protein actively interferes with photoreceptor function or viability. If this conjecture is correct, it leaves unexplained the differences in clinical course between tritanopia and retinitis pigmentosa. The nonprogressive nature of tritanopia may reflect an intrinsic difference in the physiology of rod and cone photoreceptors, a difference in the effects of the amino acid substitutions causing the two disorders, or merely the paucity of blue cones compared to rods in the human retina.

RHODOPSIN

Inherited Defects in Rod Vision

In the human retina, rods constitute >95% of the photoreceptor cells. Like the cone visual pigments, rhodopsin is highly concentrated in the photoreceptor outer segment, a specialized cilium that contains the phototransduction

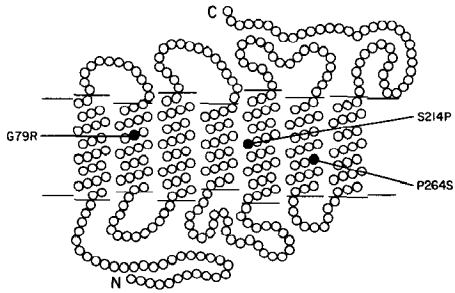

Figure 6 Schematic representation of the blue pigment showing the locations of three amino acid substitutions responsible for tritanopia. Amino acid substitutions are designated as in Figure 5. N and C denote amino- and carboxy-termini, respectively. The amino-terminus faces the extracellular space.

machinery. The 10^8 molecules of rhodopsin present in each rod outer segment are replaced every ten days throughout life.

Inherited defects in rod vision are extremely heterogeneous, both genetically and clinically (35, 67). The most benign disorder, congenital stationary nightblindness (CSNB), is characterized by an increased rod threshold, with little or no progression of signs or symptoms throughout life. Retinitis pigmentosa (RP), a more severe disorder, typically begins with an early loss of rod function or, in some individuals, both rod and cone function, followed by a slow progressive degeneration of the peripheral retina. The macula is typically spared until relatively late in the course of the disease. RP is one of the most commonly encountered retinal dystrophies, affecting approximately 1 in 4000 people in all populations examined. Pedigree analysis of RP patients in the USA reveals that in 22% the disease is due to an autosomal dominant, in 16% to an autosomal recessive, and in 9% to an X-linked gene (9). The remaining approximately 50% of patients have no family history of RP and are presumed to comprise additional instances of autosomal recessive and X-linked transmission, as well as new mutations of all types. The most severe retinal dystrophy, Leber's congenital amaurosis, is a diagnosis given to infants with blinding retinal disease for which there is no infectious or metabolic cause, and for which there are no accompanying disorders elsewhere in the body. The greater incidence of Leber's congenital amaurosis following consanguinous mating suggests that some forms are inherited in an autosomal recessive manner. Overall, the incidence is approximately one per 35,000.

Rhodopsin Mutations in Autosomal Dominant Retinitis Pigmentosa

The first indication that mutations in the rhodopsin gene might be responsible for some cases of RP came from linkage analysis in a large Irish family with autosomal dominant RP (ADRP; 56). In this family, the disease was shown to coinherit with that region of the long arm of chromosome 3 to which the rhodopsin gene had previously been mapped (61). Based upon this linkage study, a number of laboratories began to look for mutations in the rhodopsin gene in patients with ADRP. PCR amplification of coding region exons followed either by direct sequencing (23, 40), single strand conformation polymorphism gel electrophoresis (21, 22), denaturing gradient gel electrophoresis (83, 85), or hydrolink polyacrylamide gel electrophoresis (32, 41, 48) has, to date, revealed 32 different rhodopsin mutations (Figure 7). Twenty-nine of the mutations are single nucleotide substitutions (28 missense mutations and one nonsense mutation), one mutation involves the substitutions of 2 adjacent nucleotides within one codon, and two mutations are small, in-frame deletions. Most of the mutations have been tested for co-inheritance with RP in affected families, and for their presence or absence in a control population with normal vision. In each case examined, the mutation cos-

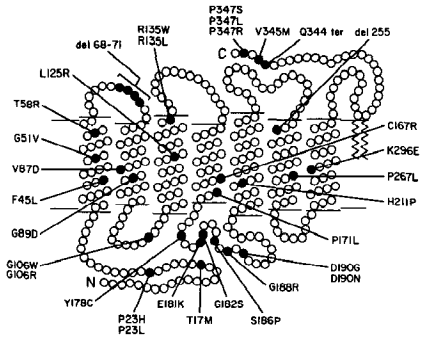

Figure 7 Schematic representation of human rhodopsin showing the locations of thirty-two mutations responsible for ADRP. Amino acid substitutions are designated as in Figure 5. N and C denote amino- and carboxy-termini, respectively. The amino-terminus faces the extracellular space. One mutation, Pro^{23}-to-His, was found in 15% of ADRP patients in the United States (23, 85), but was absent from 91 unrelated ADRP patients from Europe, Ireland, and Britain (24). All Pro^{23}-to-His patients share the same rare allele of a highly polymorphic $(CA)_n$ repeat sequence in the first intron of the rhodopsin gene, strongly suggestive of a common origin for this mutation (22). A similar analysis of the next most common mutation Pro^{347}-to-Leu, which occurs in 1–5% of ADRP patients, shows that this mutation arose independently on at least three occasions, one of which occurred de novo within a three-generation pedigree (21, 22).

egregated with RP and was absent from the control population. In three large population studies, rhodopsin mutations were found in 25–30% of individuals with ADRP (21, 41, 85). As the subjects studied thus far have been almost exclusively Caucasian, this frequency may differ in other populations.

Neutral amino acid substitutions in the rhodopsin gene appear to be extremely rare. Among 322 rhodopsin genes examined in one study, every amino acid substitution that was identified coinherited with ADRP in affected families (85). This finding rules out sequence variation within the rhodopsin gene as a cause of the reported common variation in the point of maximal sensitivity of human rod vision (3). The frequency of rhodopsin mutations in ADRP is significantly lower than the frequency of blue pigment mutations in tritanopia (25–30%, n= 350 vs 82%, n=11). This difference might reflect a larger number of rod-specific genes that are targets for mutation compared to blue cone-specific genes, or a lower threshold for pathological effects of other altered proteins in rods.

Biochemical Characteristics of Mutant Rhodopsins

The identification of rhodopsin mutations in patients with RP raises the question of the biochemical mechanism(s) responsible for the loss of rod function and subsequent retinal degeneration. In one approach to this question, the properties of 13 mutant opsins (the apoprotein of rhodopsin) found in

ADRP patients were analyzed following their production in a human embryonic kidney cell line (86). In this tissue culture expression system, wild-type human opsin is targeted to the plasma membrane where it accumulates to a concentration of approximately 3 x 10^6 molecules per cell. Addition of 11-*cis* retinal to a crude membrane fraction containing expressed wild type opsin generates a photolabile rhodopsin with the predicted absorbance properties (Figure 1). The 13 opsin mutants fall into two distinct biochemical classes. Three mutants (class I: Phe^{45}-to-Leu, Glu^{344}-to-Ter, i.e. deletion of residues 344 to 348, and Pro^{347}-to-Leu) resemble the wild type in yield, regenerability with 11-*cis* retinal, and plasma membrane localization. Ten mutants (class II: Thr^{17}-to-Met, Pro^{23}-to-His, Thr^{58}-to-Arg, Val^{87}-to-Asp, Gly^{89}-to-Asp, Gly^{106}-to-Trp, Arg^{135}-to-Leu, Arg^{135}-to-Trp, Tyr^{178}-to-Cys, and Asp^{190}-to-Gly) accumulate to significantly lower concentrations, regenerate variably or not at all with 11-*cis* retinal, and are transported inefficiently to the plasma membrane, remaining partially or predominantly in the endoplasmic reticulum (ER).

The finding of a biochemical defect in 10 of 13 mutant rhodopsins (class II mutants) associated with ADRP strongly supports the genetic inference that they are responsible for the disease. The simplest interpretation of the class II biochemical phenotype is that these mutant opsins fail to fold correctly, or, once folded, are unstable. Under this interpretation, their partial or complete retention in the ER reflects an intrinsic ability of the ER to recognize and retain unfolded or improperly folded proteins (38, 51). The behavior of class II mutants closely resembles that of a large number of bovine rhodopsin mutants in which alterations were constructed in the extracellular domains (16), suggesting that many additional sites in rhodopsin may, if mutated, lead to ADRP. Many class II mutations reside near Cys^{110} or Cys^{187} (Figure 7), two conserved residues that form an essential disulfide bond in bovine rhodopsin (46, 47). These mutations may destabilize the protein by interfering with disulfide bond formation. In one class II mutant, Thr^{17}-to-Met, one of two sites of Asn-linked glycosylation (Asn-X-Ser/Thr) is eliminated. This mutation may produce rod dysfunction by a mechanism related to that associated with tunicamycin treatment, in which inhibition of Asn-linked glycosylation is accompanied by a breakdown in the orderly assembly of the outer segment (29, 30). The pathogenic mechanisms associated with class I mutants are not yet apparent. Two of three class I amino acid changes reside near opsin's carboxy terminus, a region that shows a significant clustering of mutations (Figure 7).

Of interest is the subcellular localization of the mutant rhodopsins in the photoreceptor cell. One type of cellular pathology might arise if, in the photoreceptor cell, the class II mutant opsins accumulate in the ER as they do in tissue culture. A slow death of rod photoreceptors might occur secondary to the metabolic costs associated with inefficiencies in ER function or

degradation of the mutant protein. (In the normal case, the photoreceptors are spared the costs of degrading visual pigment because outer segment degradation is carried out exclusively by the retinal pigment epithelium.) A second type of pathology might arise if some fraction of the mutant opsin is transported to the outer segment, where it could interfere with phototransduction. This last conjecture may relate to the observation of a marked decrease in the rate of dark adaptation as well as an elevation in the dark adapted threshold in many patients with ADRP (67).

Studies aimed at correlating clinical findings with the type of mutation are underway in several laboratories, and the first results of these studies suggest some degree of allele specificity to the pattern and severity of retinal dysfunction (7, 8, 26, 27, 35, 43, 49). In particular, recent measurements of dark adaptation in patients with rhodopsin gene mutations Thr^{17}-to-Met, Pro^{23}-to-His, Thr^{58}-to-Arg, Arg^{135}-to-Leu, Arg^{135}-to-Trp, and Glu^{344}-to-Ter, show greater similarity in the time course of dark adaptation between patients carrying the same mutation, as compared to those carrying different mutations (43, 49).

PERSPECTIVE

Inherited variation in human vision has long been a source of fascination for those interested in sensory mechanisms. Anomalies of color vision were accurately described two centuries ago (13), and those of rod vision, including retinitis pigmentosa, over one century ago (17). The present wealth of knowledge about these variations has been made possible by several experimentally favorable attributes of the human visual system. First, humans recognize and can accurately report abnormalities in their vision. As a result, many people with heritable visual impairments come to the attention of an ophthalmologist. Second, human visual psychophysics is a highly developed science that offers accurate, sensitive, and noninvasive tests for defining phenotypes. Third, the retina is the only part of the central nervous system that can be viewed directly. Monitoring its appearance through the ophthalmoscope makes it possible to follow at a tissue level the natural history of retinal disease. And fourth, inherited variations in the visual system rarely affect longevity or fecundity, with the result that the responsible alleles are destined to increase in the human gene pool.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the Howard Hughes Medical Institute, the National Eye Institute, and the National Retinitis Pigmentosa Foundation.

Literature Cited

1. Alpern, M. 1974. What is it that confines in a world without color? *Inv. Ophthalmol.* 13:648–74
2. Alpern, M. 1979. Lack of uniformity in colour matching. *J. Physiol.* 288:85–105
3. Alpern, M. 1987. A note on the action spectrum of human rod vision. *Vision Res.* 27:1471–80
4. Alpern, M., Moeller, J. 1977. The red and green cone visual pigments of deuteranomalous trichromacy. *J. Physiol.* 266:647–75
5. Alpern, M., Pugh, E. N. 1977. Variation in the action spectrum of erythrolabe among deuteranopes. *J. Physiol.* 266: 613–46
6. Alpern, M., Wake, T. 1977. Cone pigments in human deutan colour vision defects. *J. Physiol.* 266:595–612
7. Berson, E. L., Rosner, B., Sandberg, M. A., Dryja, T. P. 1991. Ocular findings in patients with autosomal dominant retinitis pigmentosa and a rhodopsin gene defect (pro-23-his). *Arch. Ophthalmol.* 109:92–101
8. Berson, E. L., Rosner, B., Sandberg, M. A., Weigel-DiFranco, C., Dryja, T. P. 1991. Ocular findings in patients with autosomal retinitis pigmentosa and rhodopsin proline-347-leucine. *Am. J. Ophthalmol.* 111:614–23
9. Boughman, J. A., Fishman, G. A. 1983. A genetic analysis of retinitis pigmentosa. *Br. J. Ophthalmol.* 67: 449–54
10. Boynton, R. M. 1979. *Human Color Vision*. New York: Holt, Rinehart, Winston. 438 pp.
11. Choi, O-R. B., Engel, J. D. 1988. Developmental regulation of beta-globin gene switching. *Cell* 55:17–26
12. Cohn, S. A., Emmerich, D. S., Carlson, E. A. 1989. Differences in the responses of heterozygous carriers of colorblindness and normal controls to briefly presented stimuli. *Vision Res.* 29:255–62
13. Dalton, J. 1798. Extraordinary facts relating to the vision of colours, with observations. *Mem. Lit. Philos. Soc. London* 5:28–45
14. Dartnall, H. J. A., Bowmaker, J. K., Mollon, J. D. 1983. Human visual pigments: microspectrophotometric results from the eyes of seven persons. *Proc. R. Soc. London, Ser. B* 220:115–30
15. Deeb, S. S., Lindsey, D. T., Sanocki, E., Winderickx, J., Teller, D. Y., Motulsky, A. G. 1992. Genotye-phenotype relationships in human red/green color vision defects: molecular and psychophysical studies. *Am. J. Hum. Genet.* In press
16. Doi, T., Molday, R. S., Khorana, H. G. 1990. Role of the intradiscal domain in rhodopsin assembly and function. *Proc. Natl. Acad. Sci. USA* 87:4991–95
17. Donders, F. C. 1857. Beitrage zur pathologischen Anatomie des Auges: Pigmentbildung in der Netzhaut. *Graefes Arch. Clin. Exp. Ophthalmol.* 3:139–50
18. Donders, F. C. 1880. Remarks on colours and colour blindness. *Br. Med. J.* 2:767–69
19. Drummond-Borg, M., Deeb, S. S., Motulsky, A. G. 1988. Molecular basis of abnormal color vision: a family with three types of color vision defects. *Am. J. Hum. Genet.* 43:675–83
20. Drummond-Borg, M., Deeb, S. S., Motulsky, A. G. 1989. Molecular patterns of X chromosome-linked color vision genes among 134 men of European ancestry. *Proc. Natl. Acad. Sci. USA* 86:983–87
21. Dryja, T. P., Hahn, L. B., Cowley, G. S., McGee, T. L., Berson, E. L. 1991. Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 88:9370–74
22. Dryja, T. P., McGee, T. L., Hahn, L. B., Cowley, G. S., Olsson, J. E., Reichel, E., et al. 1990. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N. Engl. J. Med.* 323: 1302–7
23. Dryja, T. P., McGee, T. L., Reichel, E., Hahn, L. B., Cowley, G. S., et al. 1990. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* 343:364–66
24. Farrar, G. J., Kenna, P., Redmond, R., McWilliam, P., Bradley, D. G., et al. 1990. Autosomal dominant retinitis pigmentosa: absence of the rhodopsin proline to histidine (codon 23) in pedigrees from Europe. *Am. J. Hum. Genet.* 47:941–45
25. Feil, R., Aubourg, P., Helig, R., Mandel, J. L. 1990. A 195-kb cosmid walk encompassing the human Xq28 color

vision pigment genes. *Genomics* 6:367–73
26. Fishman, G. A., Stone, E. M., Gilbert, L. D., Kenna, P., Sheffield, V. C. 1991. Ocular findings associated with a rhodopsin gene codon 58 transversion mutation in autosomal dominant retinitis pigmentosa. *Arch. Ophthalmol.* 109: 1387–93
27. Fishman, G. A., Stone, E. M., Sheffield, V. C., Gilbert, L. D., Kimura, A. E. 1992. Ocular findings associated with rhodopsin gene codon 17 and codon 182 transition mutations in dominant retinitis pigmentosa. *Arch. Ophthalmol.* 110:54–62
28. Fleischman, J. A., O'Donnell, F. E. 1981. Congenital X-linked incomplete achromatopsia: evidence for slow progression, carrier fundus findings, and possible genetic linkage with glucose-6-phosphate dehydrogenase locus. *Arch. Ophthalmol.* 99:468–72
29. Fliesler, S. J., Rapp, L. M., Hollyfield, J. G. 1984. Photoreceptor-specific degeneration caused by tunicamycin. *Nature* 311:575–77
30. Fliesler, S. J., Rayborn, M. E., Hollyfield, J. G. 1985. Membrane morphogenesis in retinal rod outer segments: inhibition by tunicamycin. *J. Cell Biol.* 100:574–87
31. Francois, J. 1961. *Heredity in Ophthalmology*. St Louis: Mosby. 731 pp.
32. Gal, A., Artlich, A., Ludwig, M., Niemeyer, G., Olek, K., et al. 1991. Pro347-arg mutation of the rhodopsin gene in autosomal dominant retinitis pigmentosa. *Genomics* 11:468–70
33. Grutzner, P., Born, G., Hemminger, H. J. 1976 Coloured stimuli within the central visual field of carriers of dichromatism. *Mod. Probl. Ophthalmol.* 17:147–50
34. Hays, T. R., Lin, S. H., Eyring, H. 1980). Wavelength regulation in rhodopsin: effects of dipoles and amino acid side chains. *Proc. Natl. Acad. Sci. USA* 77:6314–18
35. Heckenlively, J. R. 1988. *Retinitis Pigmentosa*. Philadelphia: Lippincott. 269 pp.
36. Heckenlively, J. R., Rodriguez, J. A., Daiger, S. P. 1991. Autosomal dominant sectoral retinitis pigmentosa: two families with transversion mutation in codon 23 of rhodopsin. *Arch. Ophthalmol.* 109:84–91
37. Hess, R. F., Mullen, K. T., Sharpe, L. T., Zrenner, E. 1989. The photoreceptors in atypical achromatopsia. *J. Physiol.* 417:123–49
38. Hobbs, H. H., Russell, D. W., Brown, M. S., Goldstein, J. L. 1990. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu. Rev. Genet.* 24:133–70
39. Ibbotson, R. E., Hunt, D. M., Bowmaker, J. K., Mollon, J. D. 1992. Sequence divergence and copy number of the middle- and long-wave photopigment genes in old world monkeys. *Proc. R. Soc. London, Ser. B* 247:145–54
40. Inglehearn, C. F., Bashir, R., Lester, D. H., Jay, M., Bird, A. C., Bhattacharya, S. S. 1991. A 3-bp deletion in the rhodopsin gene in a family with autosomal dominant retinitis pigmentosa. *Am. J. Hum. Genet.* 48:26–30
41. Inglehearn, C. F., Keen, T. J., Bashir, R., Jay, M., Fitzke, F., et al. 1992. A completed screen for mutations of the rhodopsin gene in a panel of patients with autosomal dominant retinitis pigmentosa. *Hum. Mol. Genet.* 1:41–45
42. Jacobs, G. H., Neitz, J. 1987. Inheritance of color vision in a new world monkey (Saimiri sciureus). *Proc. Natl. Acad. Sci. USA* 84:2545–49
43. Jacobson, S. G., Kemp, C. M., Sung, C-H., Nathans, J. 1991. Retinal function and rhodopsin levels in autosomal dominant retinitis pigmentosa with rhodopsin mutations. *Am. J. Ophthalmol.* 112: 256–71
44. Jorgensen, A. L., Deeb, S. S., Motulsky, A. G. 1990. Molecular genetics of X chromosome-linked color vision among populations of African and Japanese ancestry: high frequency of a shortened red pigment gene among Afro-Americans. *Proc. Natl. Acad. Sci. USA* 87: 6512–16
45. Kalmus, H. 1955. The familial distribution of congenital tritanopia with some remarks on some similar conditions. *Ann. Hum. Genet.* 20:39–56
46. Karnik, S. S., Khorana, H. G. 1990. Assembly of functional rhodopsin requires a disulfide bond between cysteine residues 110 and 187. *J. Biol. Chem.* 265:17520–24
47. Karnik, S. S., Sakmar, T. P., Chen, H. -B., Khorana, H. G. 1988. Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. *Proc. Natl. Acad. Sci. USA* 85:8459–63
48. Keen, T. J., Inglehearn, C. F., Lester, D. H., Bashir, R., Jay, M., et al. 1991. Autosomal dominant retinitis pigmentosa: four new mutations in rhodopsin, one of them in the retinal attachment site. *Genomics* 11:199–205
49. Kemp, C. M., Jacobson, S. G., Roman,

A. J., Sung, C-H., Nathans, J. 1992. Abnormal rod dark adaptation in autosomal dominant retinitis pigmentosa with proline-23-histidine rhodopsin mutation. *Am. J. Ophthalmol.* 113:165–74
50. Koenig, A., Dieterici, C. 1893. Die Grundempfindungen in normalen und anomalen Farbensystemen und ihre Intensitätsverteilung im Spektrum. *Z. Psychol. Physiol. Sinnesorg.* 4:241–347
51. Klausner, R. D., Sitia, R. 1990. Protein degradation in the endoplasmic reticulum. *Cell* 62:611–14
52. Krill, A. E., Smith, V. C., Pokorny, J. 1971. Further studies supporting the identity of congenital tritanopia and hereditary dominant optic atrophy. *Inv. Ophthalmol.* 10:457–65
53. Levy, M. 1905. Uber einen Fall von angeborener beiderseitiger Tritanopie (Blaublindheit). *Graefes Arch. Ophthalmol.* 62:464–80
54. Maxwell, J. C. 1860. On the theory of compound colours, and the relations of the colours of the spectrum. *Philos. Trans. R. Soc. London* 150:57–84
55. McNaughton, P. 1990. Light response of vertebrate photoreceptors. *Physiol. Rev.* 70:847–83
56. McWilliam, P., Farrar, G. J., Kenna, P., Bradley, D. G., Humphries, M. M., et al. 1989. Autosomal dominant retinitis pigmentosa (ADRP): localization of an ADRP gene to the long arm of chromosome 3. *Genomics* 5:619–22
57. Merbs, S. L., Nathans, J. 1992. Absorption spectra of human cone pigments. *Nature* 356:433–35
57a. Merbs, S. L., Nathans, J. 1992. Absorption spectra of the hybrid pigments responsible for anomalous color vision. *Science*. In press
58. Miyake, Y., Yagasaki, K., Ichikawa, H. 1985. Differential diagnosis of congenital tritanopia and dominantly inherited juvenile optic atrophy. *Arch. Ophthalmol.* 103:1496–501
59. Nathans, J., Davenport, C. M., Maumenee, I. H., Lewis, R. A., Hejtmancik, J. F., et al. 1989. Molecular genetics of human blue cone monochromacy. *Science* 245:831–38
60. Nathans, J., Hogness, D. S. 1984. Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc. Natl. Acad. Sci. USA* 81:4851–55
61. Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B., Hogness, D. S. 1986. Molecular genetics of inherited variation in human color vision. *Science* 232:203–10
62. Nathans, J., Thomas, D., Hogness, D. S. 1986. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science* 232: 193–202
63. Neitz, J., Jacobs, G. H. 1986. Polymorphism of the long-wavelength cone in normal human color vision. *Nature* 323:623–25
64. Neitz, J., Jacobs, G. H. 1990. Polymorphism in normal human color vision and its mechanism. *Vision Res.* 30:621–36
65. Neitz, J., Neitz, M., Jacobs, G. H. 1989. Analysis of fusion gene and encoded photopigment of colour-blind humans. *Nature* 342:679–82
66. Neitz, M., Neitz, J., Jacobs, G. H. 1991. Spectral tuning of pigments underlying red-green color vision. *Science* 252:971–74
67. Newsome, D. 1988. *Retinal Dystrophies and Degenerations*. New York: Raven. 382 pp.
68. Oprian, D. D., Asenjo, A. B., Lee, N., Pelletier, S. L. 1991. Design, chemical synthesis, and expression of genes for the three human color vision pigments. *Biochemistry* 30:11367–72
69. Padmos, P., van Norren, D., Jaspers-Faijer, J. W. 1978. Blue cone function in a family with an inherited tritan defect, tested with electroretinography and psychophysics. *Invest. Ophthalmol. Vis. Sci.* 117:436–41
70. Piantanida, T. P., Sperling, H. G. 1973. Isolation of a third chromatic mechanism in the protanomalous observer. *Vision Res.* 13:2033–47
71. Piantanida, T. P., Sperling, H. G. 1973. Isolation of a third chromatic mechanism in the deuteranomalous observer. *Vision Res.* 13:2049–58
72. Pickford, R. W. 1957. A practical anomaloscope for testing colour vision and colour blindness. *Br. J. Physiol. Optics* 14:2–26
73. Pokorny, J., Smith, V. C., Verriest, G., Pinckers, A. J. L. G. 1979. *Congenital and Acquired Color Vision Defects*. New York: Grune & Stratton. 409 pp.
74. Pokorny, J., Smith, V. C., Went, L. N. 1981. Color matching in autosomal dominant tritan defect. *J. Opt. Soc. Am.* 71:1327–34
75. Post, R. H. 1962. Population differences in red and green color vision deficiency: a review, and a query on selection relaxation. *Eugen. Q.* 9:131–46
76. Rayleigh, L. 1881. Experiments on colour. *Nature* 25:64–66
77. Reichel, E., Bruce, A. M., Sandberg,

M. A., Berson, E. 1989. An electroretinographic and molecular genetic study of X-linked cone degeneration. *Am. J. Ophthalmol.* 108:540–47
78. Reitner, A., Sharpe, L. T., Zrenner, E. 1991. Is colour vision possible with only rods and blue-sensitive cones? *Nature* 352:798–800
79. Rushton, W. A. H. 1972. Visual pigments in man. In *Handbook of Sensory Physiology: Photochemistry of Vision,* ed. H. J. A. Dartnall, 7:364–94. Berlin: Springer-Verlag. 810 pp.
80. Rushton, W. A. H., Baker, H. D. 1964. Red/green sensitivity in normal color vision. *Vision Res.* 4:75–85
81. Rushton, W. A. H., Powell, D. S., White, K. D. 1973. Pigments in anomalous trichromats. *Vision Res.* 13: 2017–31
82. Schnapf, J. L., Kraft, T. W., Baylor, D. A. 1987. Spectral sensitivity of human cone photoreceptors. *Nature* 325:439–41
83. Sheffield, V. C., Fishman, G. A., Beck, J. S., Kimura, A. E., Stone, E. M. 1991. Identification of novel rhodopsin mutations associated with retinitis pigmentosa by GC-clamped denaturing gradient gel electrophoresis. *Am. J. Hum. Genet.* 49:699–706
84. Stryer, L. 1986. Cyclic GMP cascade of vision. *Annu. Rev. Neurosci.* 9:87–119
85. Sung, C-H., Davenport, C. M., Hennessey, J. C., Maumenee, I. H., Jacobson, S. G., et al. 1991. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 88:6481–85
86. Sung, C-H., Schneider, B., Agarwal, N., Papermaster, D. S., Nathans, J. 1991. Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 88:8840–44
87. van Heel, L., Went, L. N., van Norren, D. 1980. Frequency of tritan disturbances in a population study. *Color Vision Defic.* 5:256–60
88. Vollrath, D., Nathans, J., Davis, R. W. 1988. Tandem array of human visual pigment genes at Xq28. *Science* 240: 1669–72
89. Waaler, G. H. M. 1967. Heredity of two types of normal color vision. *Nature* 215:406
90. Wald, G. 1967. Blue-blindness in the normal fovea. *J. Opt. Soc. Am.* 57: 1289–301
90a. Wang, Y., Macke, J. P., Merbs, S. L., Zack, D. J., Klaunberg, B., et al. 1992. A locus control region adjacent to the human red and green pigment genes. *Neuron.* In press
91. Weatherall, D. J., Clegg, J. B., Higgs, D. R., Wood, W. G. 1989. The hemoglobinopathies. In *The Metabolic Basis of Inherited Disease,* ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, pp. 2281–39. New York: McGraw-Hill
92. Weitz, C. J., Miyake, Y., Shinzato, K., Montag, E., Zrenner, E., et al. 1992. Human tritanopia associated with two amino acid substitutions in the blue sensitive opsin. *Am. J. Hum. Genet.* 50:498–507
93. Weitz, C. J., Went, L. N., Nathans, J. 1992. Human tritanopia associated with a third amino acid substitution in the blue-sensitive visual pigment. *Am. J. Hum. Genet.* In press
94. Went, L. N., Pronk, N. 1985. The genetics of tritan disturbances. *Hum. Genet.* 69:255–62
95. Williams, A. J., Hunt, D. M., Bowmaker, J. K., Mollon, J. D. 1992. The polymorphic photopigments of the marmoset: spectral tuning and genetic basis. *EMBO J.* 51:444–46
96. Winderickx, J., Lindsey, D. T., Sanocki, E., Teller, D. Y., Motulsky, A. G., Deeb, S. S. 1992. Polymorphism in red photopigment underlies variation in colour matching. *Nature* 356:431–33
97. Winderickx, J., Sanocki, E., Lindsey, D. T., Teller, D. Y., Motulsky, A. G., Deeb, S. S. 1992. Defective color vision associated with a missense (cys-203-arg) mutation in the human green visual pigment gene. *Nature Genet.* 11:2039–45
98. Wright, W. D. 1952. The characteristics of tritanopia. *J. Opt. Soc. Am.* 42:509–21
99. Wyszecki, G., Stiles, W. S. 1982. *Color Science: Concepts and Methods, Quantitative Data and Formulae.* New York: Wiley. 950 pp.

Annual Review of Genetics
Volume 26, 1992

CONTENTS