Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

Visual Pigments and Molecular Genetics of Color Blindness

J. K. Bowmaker

Red/green color blindness, found in ~1 in 15 men, is caused by the expression of hybrid genes coding for visual pigments. Spectral information from site-directed mutagenesis and recombinant expression has led to the possibility of correlating individual genotypes with psychophysical measurements of the severity of the deficiency.

Color vision in humans is trichromatic; that is, we possess three spectrally distinct classes of photoreceptor cells, cones, arranged in a regular mosaic across the back of the retina. Each class of cone contains a visual pigment that is maximally sensitive to a different part of the spectrum: red or long-wave sensitive (LWS), green or middle-wave sensitive (MWS), and blue or short-wave sensitive (SWS). Recent advances in molecular genetics have given us

"Color vision in humans is trichromatic. . . ."

J. K. Bowmaker is in the Dept. of Visual Science, Institute of Ophthalmology, University College London, Bath St., London EC1V 9EL, UK.

0886-1714/98 5.00 @ 1998 Int. Union Physiol. Sci./Am.Physiol. Soc.

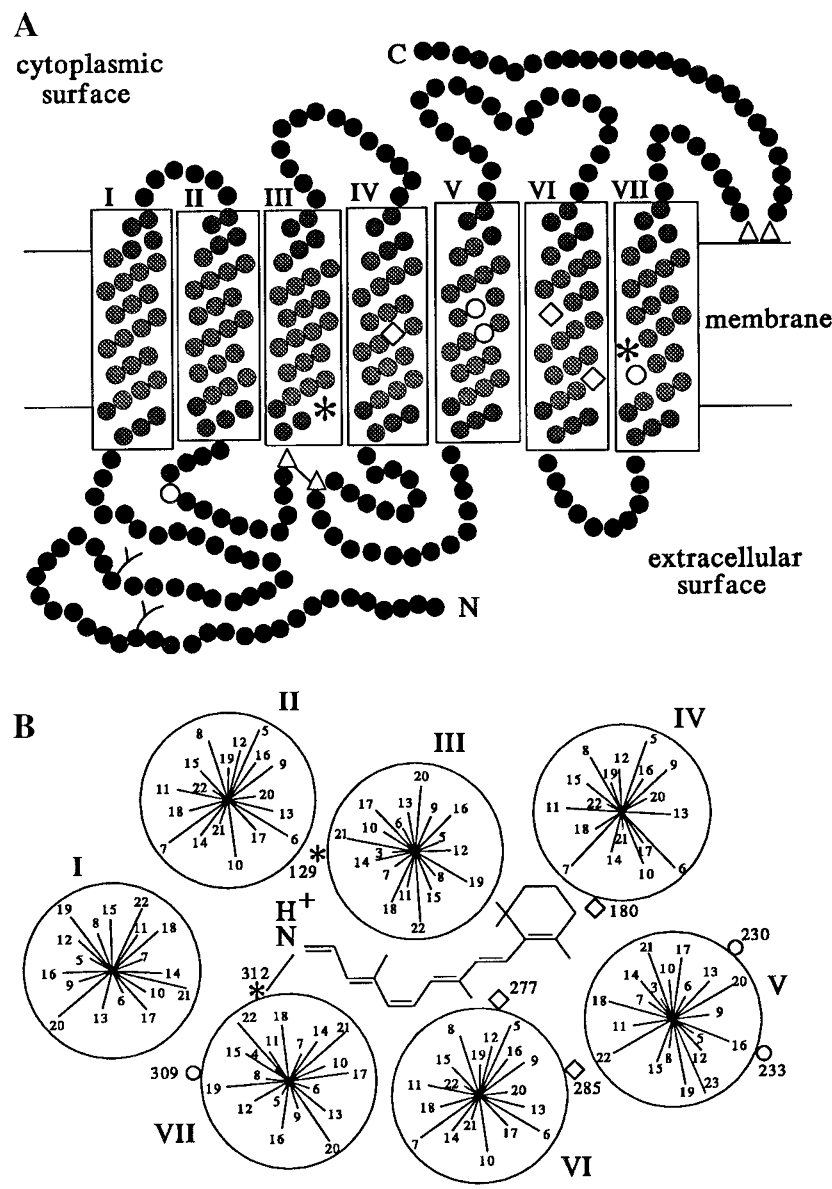

FIGURE 1. *A*: schematic diagram of the organization within lipid bilayer of the disk membrane of opsins from human long-wave-sensitive (LWS) and middle-wave-sensitive (MWS) cone pigments. Opsins consist of 364 amino acids, including 2 glycosylation sites near the NH_2 terminus (N) and a series of phosphorylation sites near the COOH terminus (C). Two cysteines (△) adjacent to the extracellular end of helix III form a disulfide bridge essential for correct folding of the molecule, and 2 further cysteines (△) on the cytoplasmic surface of the membrane are palmitoylated, thus forming a 4th cytoplasmic loop. Retinal is attached via a protonated Schiff's base to lysine 312 (designated by * in helix VII), which has a counterion, glutamate at 129 (designated by * in helix III). Sites 180, 277, and 285 (◇ in helix IV and VI) are hydroxyl/nonhydroxyl amino acid exchanges that primarily determine the spectral tuning of the LWS and MWS pigments. Additional sites (○) 116 (first extracellular loop), 230 and 233 (helix V), and 309 (helix VII) are also involved to a much lesser extent in the spectral tuning of these pigments. *B*: 3-dimensional model of the LWS and MWS pigments showing relative positions of the 7 α-helical transmembrane regions (2) and location of retinal within the helical palisade. Each circle represents an α-helix containing 26 amino acids, and each radiating line within the circle represents an amino acid. Position of each line indicates location of the amino acid around the circumference of the helix, whereas length of line indicates the depth of the amino acid within the helix. Helix III is somewhat buried within the palisade. Retinal is bound via a protonated Schiff's base (NH^+) to lysine 312 (*), position 11 in helix VII, which is stabilized by the counterion, glutamate 129 (*), position 3 in helix III. Orientation of retinal is purely schematic. The 3 sites (◇) principally involved in tuning between the LWS and MWS pigments are located at position 14 in helix IV and positions 12 and 20 in helix VI. ○, Three additional tuning sites located in helices.

an insight into the structural features of these pigments, their evolutionary history, and the modifications that occur in those people who have inherited color vision deficiencies.

Structure of opsin

Visual pigments belong to a very large family of structurally similar transmembrane proteins that

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

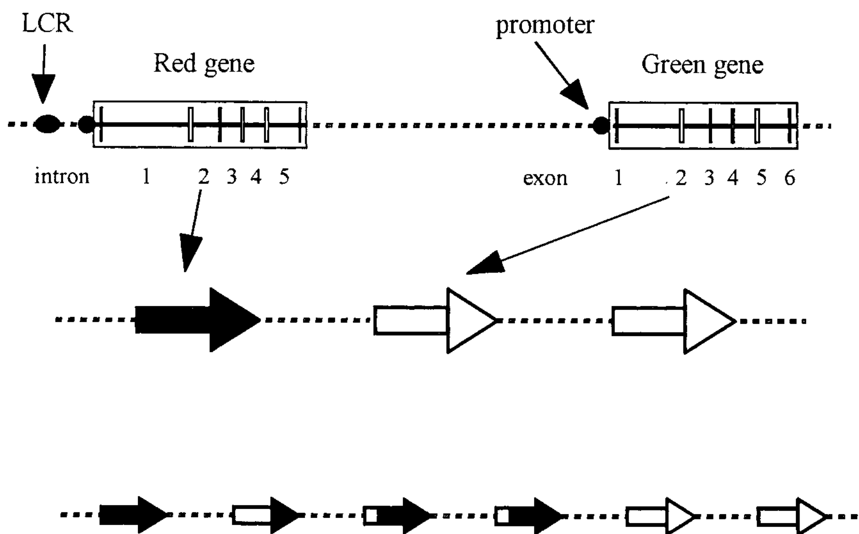

FIGURE 2. Diagrammatic representations of the L/G gene array on the q arm of the X chromosome. *Top*: relative positions and structure of the red and green genes. Lengths of the 6 exons, 5 introns, and intergene regions are proportional. Locus control region (LCR) can activate one of the promoter regions just upstream of each gene. *Middle*: typical gene array of a normal observer, as suggested by Nathans et al. (9), with 1 R gene followed by 2 G genes. *Bottom*: more complex gene array of a normal observer, as suggested by Neitz et al. (12), which includes a number of hybrid genes that if expressed would produce a long-wave-sensitive pigment.

act as receptor molecules in a wide range of cell types: all function through the activation of a G protein that binds guanosine diphosphate (2). Opsin, the protein component of visual pigments, consists of a single polypeptide chain that in primates and humans is composed of either 348 or 364 amino acids and that forms seven hydrophobic regions spanning the membrane, linked by extramembrane hydrophilic loops (9) (Fig. 1*A*). The hydrophobic transmembrane segments, comprising ~50% of the molecule, are formed of α-helices, each composed of ~26 amino acids of which only the central 18 are embedded within the membrane (2), whereas the remaining hydrophilic regions, constituting ~25% of the molecule on each side of the membrane, are presumed to be straight chains. As in all G protein-coupled receptors, the NH_2 terminus is extracellular (the luminal side in the closed disks of rod outer segments), whereas the COOH terminus is intracellular. The seven membrane helices form a bundle or palisade within the membrane that diverges toward the extracellular surface, creating a cavity that acts as a ligand-binding pocket. Although the opsin polypeptides vary in length, the transmembrane helices and the cytoplasmic loops are highly conserved, with the difference in length restricted to the extracellular tail.

The different opsins share a number of features along with other heptahelical G protein-coupled receptors (5) (Fig. 1*A*). There are two glycosylation sites near the NH_2 terminus on the extracellular side and a series of phosphorylation sites near the COOH terminus on the cytoplasmic side. Also, regions of the cytoplasmic loops linking the helices are involved in interacting with the membrane-bound G protein. In addition, there are two highly conserved cysteine residues in the first and second extracellular loops that form a disulfide bridge thought to be essential for the correct folding and formation of the molecule and two further cysteines in the carboxyl tail that are palmitoylated, anchoring the tail and forming a fourth cytoplasmic loop (Fig. 1*A*).

In visual pigments, the ligand, retinal, is bound into the molecular pocket and must be activated by light, whereas, in other membrane receptors, the binding of the ligand itself (for example, epinephrine in the β-adrenergic receptors) activates the receptor molecule. Because all mammalian visual pigments contain retinal but have absorption or sensitivity maxima (λ_{max}) ranging from ~360 nm in the near ultraviolet, as found in mice and guinea pigs, to ~565 nm in the red, as found in primates and humans, the precise λ_{max} of a given pigment will be determined by the amino acid sequence of the opsin and the interaction of specific amino acids with retinal in the ligand-binding pocket.

"Opsin. . . consists of a single polypeptide chain. . . ."

Opsin genes

The three cone visual pigments in humans have λ_{max} of ~420, 530, and 562 nm (3), and each of the three opsins is coded by a distinct opsin gene (9). The gene coding for the SWS cone opsin (S gene) lies on chromosome 7, whereas the genes coding for the MWS and LWS cone opsins lie in a head-to-tail tandem array on the q arm (long arm) of the X chromosome. The L and M genes are very

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

Gene	Exon sequence 2 3 4 5	Pigment λ_{max} a	b
R class			
R (S_{180})	■ ■ ■ ■	556.7	563
R (A_{180})	■ ■ ■ ■	552.4	556
	(■ □ ■ ■)		
G2R3 (S_{180})	□ ■ ■ ■	553.0	559
G2R3 (A_{180})	□ ■ ■ ■	549.6	
	(□ □ ■ ■)		
G3R4	□ □ ■ ■	548.8	555
G4R5	□ □ □ ■	544.8	551
G class			
R4G5 (S_{180})	■ ■ ■ □	536.0	538
R4G5 (A_{180})	■ ■ ■ □	531.6	
R3G4 (S_{180})	■ ■ □ □	533.3	534
R3G4 (A_{180})	■ ■ □ □	529.0	532
	(■ □ □ □)		
R2G3	■ □ □ □	529.5	532
G (S_{180})	□ □ □ □		534
	(□ ■ □ □)		
G (A_{180})	□ □ □ □	529.7	532

FIGURE 3. Absorbance maxima (λ_{max}) of long-wave-sensitive (LWS) and middle-wave-sensitive (MWS) cone pigments as expressed from exon combinations indicated. ■, Exons from R gene; □, exons from G gene; *a*, λ_{max} from Ref. 6; and *b*, λ_{max} from Ref. 1. Note that, because of the similarity of exon 3, substitution of alanine for serine at site 180 is equivalent to exchanging exon 3 of the G gene for exon 3 of the R gene, as indicated by exon sequences in parentheses.

"...substitutions located within the membrane helices... major effects on the spectral tuning...."

similar and consist of six exons (coding regions of DNA) separated by relatively long noncoding introns. In most individuals, the L and M genes are ~15.1 and 13.2 kilobases (kb) long, respectively, with the 1.9-kb difference residing solely in intron 1. The genes are separated by long noncoding regions of ~24 kb (Fig. 2).

The deduced amino acid sequence of the S opsin consists of 348 amino acids and is similar to the opsins of violet- and ultraviolet-sensitive cone pigments (with λ_{max} between ~360 and 430 nm) of other vertebrates. The LWS and MWS cone opsins are ~96% homologous and clearly belong to the same phylogenetic group of LWS opsins. Both consist of 364 amino acids and differ at only 15 sites, so that the approximate 30-nm spectral difference between the two visual pigments must be attributable to substitutions at some or all of these sites. Of these, substitutions located within the membrane helices and in close proximity to the chromophore are most likely to have major effects on the spectral tuning of the pigment. Furthermore, only replacements of charged for noncharged or polar for nonpolar amino acids are likely to have significant effects on the chromophore. Three primary sites have been implicated as the most likely candidates (9, 13): site 180 in helix IV and sites 277 and 285 in helix VI (Fig. 1), with sites 277 and 285 responsible for ~20–25 nm of the spectral difference. In the shorter-wave P530, all three sites are occupied by nonpolar residues (alanine, phenylalanine, and alanine, respectively), whereas, in the longer-wave P562, the three sites are occupied by hydroxyl-bearing polar residues with a small negative charge (serine, tyrosine, and threonine, respectively).

The head-to-tail arrangement of the L and M genes on the X chromosome (Fig. 2) and their close sequence similarity mean that this region of the chromosome is susceptible to mispairing during meiosis, leading to unequal crossing over

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

Observer		gene arrangement		Predicted spectral separation nm
	exon	2 3 4 5		
dichromat		■■■■--		0.0
very severe		■□■■--□■■■--□□□□--		
	λ_{max} [a]	552.4	553.0	0.6 [a]
	λ_{max} [b]	556	559	3 [b]
severe		■□■■--□□■■--□□□□--		1 [b] to 3.6 [a]
		■■■■--□■■■--□□□□--		3.7 [a] to 4 [b]
mild		■□■■--□□□■--□□□□--		5 [b] to 7.6 [a]
		■■■■--□□■■--□■■■--□□□□--		7.5 [a] to 8 [b]
very mild		■■■■--□■□■--□□□□--		9 [b]
		■■■■--□□□■--□□□□--		
	λ_{max} [a]	556.7	544.8	11.9 [a]
	λ_{max} [b]	563	551	12 [b]

FIGURE 4. Correlation of genotype and phenotype in deuteranomalous observers. ■, Exons from R gene; □, exons from G gene. Gene origin of exon 3 is defined by codon 180: if coding for alanine, then the exon is classified as from a G gene; if for serine then from an R gene. Values for absorbance maxima (λ_{max}) are from Ref. 6 (a) and from Ref. 1 (b). In all cases, normal G gene is not expressed. Data are from Ref. 11.

between the gene arrays. If the crossover occurs between genes, this will result in the deletion of a gene from one chromosome and its addition to the other, whereas a crossover within a gene will lead to the production of a hybrid or chimeric gene that combines regions of the L and M genes into a single gene. Such hybrid genes are thought to be responsible for anomalous color vision in humans (8).

The codons for the three amino acids primarily involved in spectral tuning are located in exon 3 (site 180) and exon 5 (sites 277 and 285). Thus, in hybrid genes composed of a mixture of L and M exons, sites 277 and 285 will remain together and the λ_{max} of the visual pigment will be largely determined by whether exon 5 is from an L gene or an M gene. Hybrid genes that contain the L exon 5 are expressed as pigments, with λ_{max} between about 545 and 557 nm (6) or 551 and 563 nm (1), whereas pigments expressed from genes containing the M exon 5 have λ_{max} between about 530 and 536 nm (6) or 532 and 538 nm (1) (Fig. 3). Some of the other sites located in exons 2 and 4 appear to cause small spectral shifts of only a few nanometers, whereas exons 1 and 6 are identical in the L and M genes and are therefore not significant in terms of spectral tuning. The degree of spectral shift resulting from single amino acid substitutions may vary, depending on the specific opsin involved, and it has been suggested that substitution of serine for alanine at site 180 causes a 2-nm red shift in the MWS cone pigment but a 7-nm red shift in the LWS cone pigment (1).

A complication that has recently become apparent is that there is a clear variation in the color matching of people with normal color vision. Measurements of color matching are obtained from an anomaloscope in which the observer is asked to match a monochromatic yellow light with a mixture of red and green lights (a Rayleigh match) (10, 15). Close inspection of anomaloscope settings for a large group of Caucasian males classified as having normal color vision shows that ~40% of them require slightly more red in their mixtures than do others. These differences are small in comparison to the large discrepancies found in color deficiencies but are nevertheless highly consistent. Analysis of the L and M genes of some of these individuals shows that there is a polymorphism in the L gene. In other words, within the normal population, more than one form of the gene coding for the LWS cone pigment exists.

"...there is a clear variation in the color matching of people with normal color vision."

This polymorphism is the result of a single nucleotide difference in the gene that changes

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

the genetic code for the amino acid at site 180. In some individuals, site 180 contains alanine, whereas in others the site is occupied by serine. As detailed above, the presence of serine at site 180 produces a visual pigment that is slightly more long-wave sensitive than one with alanine at site 180. Those individuals with serine at site 180 in their LWS cone opsin thus require less red in their color match than those with alanine and vice versa. Because of X-chromosome inactivation, women who are heterozygous for the L gene will express both forms of the LWS cone pigment in separate cone populations. This should mean that they would make a color match somewhere in between their male counterparts; however, because they possess four cone pigments, there is the possibility that they might have a form of tetrachromatic color vision.

With such a detailed understanding of the gene structure underlying the λ_{max} of these cone pigments, it should be relatively straightforward to correlate the gene complement of an individual with his or her color vision. Unfortunately, this has proved more difficult than expected. First, the tandem array of genes is normally more complex than one L gene followed by one M gene, but the exact arrangement of genes in the array has proved somewhat controversial. Nathans et al. (9) proposed that, although the first gene in the array is always L, one to four M genes follow, with most individuals having two M genes (Fig. 2). More recently, Neitz et al. (12) have suggested that the array is even longer, with individuals having as many as nine genes of which many may be hybrid. Some individuals have more than one L gene at the beginning of the array sequence, with some of the L genes being hybrid and containing at least exon 5 of the normal L gene (Fig. 2). Indeed, evidence exists for individuals who have only a single L gene that is a hybrid but who yet exhibit normal color vision (12).

"The most common form of anomalous color vision is deuteranomaly. . . ."

However, irrespective of the complexity of the gene array, it is thought that in any given cone only one of the opsin genes is expressed. The control of expression is governed by a locus control region (LCR) some 4 kb upstream of the first gene in the array that may link with and activate one of the promoter regions located close to and upstream of each gene (Fig. 2). Exactly which gene in the array is promoted will depend on the degree of folding or looping of the arm of the chromosome that will bring the LCR into contact with a single promoter region (14).

The critical role of the LCR in expression of the L and G genes has been demonstrated in a subset of individuals who exhibit a very rare form of color deficiency, blue cone monochromasy. In this deficiency, affecting only ~0.001% of the population, the LWS and MWS cone pigments are absent and vision is based only on SWS cones and rods. An analysis of the R/G gene arrays of these individuals (7) has shown deletions of various lengths upstream of the array, but which in all cases include the LCR. In some other sufferers of blue cone monochromasy, the cause could be attributed to a combination of gene deletions and point mutations in the genes that lead to nonfunctional pigments.

Red/green color deficiencies

Color deficiency is a relatively common phenomenon for ~8% of men, but only ~0.5% of women exhibit some form of abnormality in their color vision. The vast majority of these deficiencies are expressed as differences in the perceptions of reds and greens, with ~1 in 15 men exhibiting a red/green color deficiency. The sex-linked expression of these color deficiencies is directly related to the location of the L and M gene array on the X chromosome.

Red/green deficiencies fall into two basic classes: dichromats, who are completely red/green color blind, and anomalous trichromats, who exhibit a reduced sensitivity to either red or green. Dichromats possess only a single visual pigment in the middle- to long-wave spectral region, normally either the typical MWS (protanopes) or LWS pigment (deuteranopes). One of the earliest detailed descriptions of dichromacy was provided more than 200 years ago by the chemist John Dalton. Both he and his brother confused bright reds (scarlet) with deep greens and pinks with blue, and Dalton believed that the problem was a result of the vitreous humor, the fluid filling the eye, being colored blue. To verify his theory, Dalton asked that after his death his eyes be examined. However, at the autopsy, the humors of the eye were found to be perfectly transparent, with the lens showing the normal yellow coloration expected of a septuagenarian. Recently, it has been possible to isolate and sequence Dalton's opsin genes (4) and establish that his dichromacy was due to a complete absence of the M gene.

The most common form of anomalous color vision is deuteranomaly, present in ~4% of men, in which the observer has reduced sensitivity to green. Protanomaly, in which more red is required in the anomaloscope match, is less common, occurring in only ~1% of men. However, within both forms of anomaly, there is considerable variation. Some individuals exhibit only a mild form, whereas in others it can be severe and, in addition, the precision of their anomaloscope matches may also vary, with

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017

some observers able to make very precise matches, whereas others will accept a wide range.

A simple genetic explanation for both forms of anomaly is that the retina expresses the normal S gene, either the normal L or M gene, and then a hybrid gene that codes for a visual pigment with λ_{max} intermediate between that of the normal LWS and MWS pigments. In the case of deuteranomaly, for instance, the opsin of the anomalous pigment will be expressed from a hybrid gene that contains at least exon 5 of the L gene, yielding λ_{max} close to that of the normal LWS pigment, with the precise λ_{max} of the pigment being determined by the degree of hybridization of the remaining exons (Fig. 3). The reverse situation would occur in protanomaly. Recent analysis of 16 deuteranomalous observers (11) has demonstrated a relatively clear correlation between the severity of the anomaly and the complement of hybrid genes. The correlation is based on the predicted separation of the two visual pigments belonging to the LWS class of pigments as determined from the gene complement. The assumption is that in mild deuteranomaly the two pigments will be spectrally separated to a significantly greater degree than in more severe forms.

The correlation between genotype and phenotype is complex, however. For example, an individual with a very mild form of deuteranomaly (Fig. 4) may possess a normal L gene, a hybrid gene containing exon 5 of the L gene, and a normal G gene. If the normal L gene and the hybrid gene are expressed, then the spectral separation of the two pigments would be ~12 nm. In contrast, in the very severe form (Fig. 4), the two pigments from the LWS group would have λ_{max} separated by a maximum of only 3 nm. A surprising finding in these results is that 13 of the 16 subjects possess normal G genes, but, apparently, these are not expressed. At present, as is clear from Figs. 3 and 4, it is not possible to determine the phenotype from the genotype and vice versa, although the phenotype may give indications of the spectral separation of the two pigments and therefore limit the number of possible genes that may be expressed.

There are additional difficulties in attempting to correlate phenotype and genotype. A question that remains to be answered is whether the visual pigments expressed by hybrid genes behave in an identical manner to that of normal pigments. Is a hybrid opsin expressed to a greater or lesser extent than a normal opsin? Is its incorporation into the membranes of the cone outer segments diminished or enhanced? Either of these factors may contribute to the effective photon catch of a given class of cone, which may in turn have an effect on color matching. For any individual, although an idea of the number and type of genes in the R/G gene array can be arrived at and the first gene in the array can be determined (because of its unique upstream region), the order of the remaining genes and which single gene of the array is expressed in any given class of cone are very much open to question.

"...it is not possible to determine the phenotype from the genotype...."

References

1. Asenjo, A. B., J. Rim, and D. D. Oprian. Molecular determinants of human red/green color discrimination. *Neuron* 12: 1131–1138, 1994.
2. Baldwin, J. M. Structure and function of receptors coupled to G proteins. *Curr. Opin. Cell Biol.* 6: 180–190, 1994.
3. Dartnall, H. J. A., J. K. Bowmaker, and J. D. Mollon. Human visual pigments: microspectrophotometric results from the eyes of seven persons. *Proc. R. Soc. Lond. B Biol. Sci.* 220: 115–130, 1983.
4. Hunt, D. M., K. S. Dulai, J. K. Bowmaker, and J. D. Mollon. The chemistry of John Dalton's color blindness. *Science* 267: 984–988, 1995.
5. Liggett, S. Functional properties of human β_2-adrenergic receptor polymorphisms. *News Physiol. Sci.* 10: 265–273, 1995.
6. Merbs, S. L., and J. Nathans. Absorption spectra of the hybrid pigments responsible for anomalous color vision. *Science* 258: 464–466, 1992.
7. Nathans, J., I. H. Maumenee, E. Zrenner, B. Sadowski, L. T. Sharpe, R. A. Lewis, E. Hansen, T. Rosenberg, M. Schwartz, J. R. Heckenlively, E. Traboulsi, R. Klingaman, N. T. BechHansen, G. R. LaRoche, R. A. Pagon, W. H. Murphey, and R. G. Weleber. Genetic heterogeneity among blue-cone monochromats. *Am. J. Hum. Genet.* 53: 987–1000, 1993.
8. Nathans, J., T. P. Piantanida, R. L. Eddy, T. B. Shows, and D. S. Hogness. Molecular genetics of inherited variations h1 human color vision. *Science* 232: 203–210, 1986.
9. Nathans, J., D. Thomas, and D. S. Hogness. Molecular genetics of human color vision: the genes encoding blue, green and red pigments. *Science* 232: 193–203, 1986.
10. Neitz, J., and G. H. Jacobs. Polymorphism of the long-wavelength cone in normal human colour vision. *Nature* 323: 623–625, 1986.
11. Neitz, J., M. Neitz, and P. M. Kainz. Visual pigment gene structure and the severity of color vision defects. *Science* 274: 801–804, 1996.
12. Neitz, M., J. Neitz, and A. Grishok. Polymorphism in the number of genes encoding longwavelength-sensitive cone pigments among males with normal color vision. *Vision Res.* 35: 2395–2407, 1995.
13. Neitz, M., J. Neitz, and G. H. Jacobs. Spectral tuning of pigments underlying red-green color vision. *Science* 252: 971–974, 1991.
14. Winderickx, J., L. Battisti, A. G. Motulsky, and S. S. Deeb. Selective expression of human X chromosome-linked green opsin genes. *Proc. Natl. Acad. Sci. USA* 89: 9710–9714, 1992.
15. Winderickx, J., D. T. Lindsey, E. Sanocki, D. Y. Teller, A. G. Motulsky, and S. S. Deeb. Polymorphism in red photopigment underlies variation in colour matching. *Nature* 356: 431–433, 1992.

Downloaded from http://physiologyonline.physiology.org/ by 10.220.33.6 on October 18, 2017