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New Aspects of an Old Theme: The Genetic Basis of Human Color Vision

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Most of the readers of this journal can perceive the full range of colors in the visible spectrum and, thus, are able to discriminate among the color-coded bases of the DNA graphic on the cover and enjoy the intrinsic beauty of multicolor FISH micrographs and the brightness of dye-labeled sequencing fragments on the screen of an automatic DNA sequencer. We appreciate the palette of impressionist painters, and we deal freely with yellow submarines, green traffic lights, and red emergency buttons. Vision is the dominant sense in humans, and for most of us life is color coded.

Color vision and its deficiencies are long-standing concerns of human geneticists, but it was only with the molecular characterization of common forms of color-vision abnormalities (beginning in the late 1980s) that mechanisms emerged to explain well-documented clinical findings. Now, new questions have arisen about the evolution and structural variability of the pigment genes and the regulation of their expression. In recent studies, the identification of mutations in the gene that encodes a cGMP-gated cation channel has resolved the molecular basis of complete color blindness, thereby extending our knowledge of color vision and revealing significant analogies among some very different sensory signal transduction systems.

Basic Vision

Our vision relies on the ability to perceive a narrow window of electromagnetic radiation, and our sense of color depends on the ability to discriminate among different wavelength stimuli. Two types of photoreceptors—rods and cones, identified in accordance with the morphological appearance of their light-sensitive tips—are present in the vertebrate retina. Rods function at dim light and are effective in the detection of even single photons, whereas cones exhibit lower threshold sensitivity (daylight vision) but can adapt to a wide range of light intensities. The ability to resolve closely juxtaposed images, which determines our visual acuity, is determined by the density of cone photoreceptors in the central retina with optimized postreceptoral neuronal projections.

Comparative physiological and biochemical analyses have shown that the molecular mechanism of phototransduction and the components involved are essentially the same in rods and cones: an excitable visual pigment or opsin is activated by light, which causes cis-trans isomerization of the attached chromophore. The photoactivated pigment activates the G-protein transducin, which in turn activates a cGMP-phosphodiesterase that causes cGMP concentrations to decline sharply. Plasma membrane cGMP-gated cation channels then close, leading to a local membrane hyperpolarization signal, which is transmitted to the photoreceptor synapse. Although functionally analogous, rods and cones use different protein isoforms (the products of a distinct set of genes) in most of these steps including the photopigments (for a review, see Yau 1994).

Rods and cones express different types of photopigments. In humans, the rod pigment, rhodopsin, exhibits a peak absorption at $\lambda \approx 498$ nm, whereas the three types of cones present in the normal human retina express pigments with maximal absorption at $\lambda \approx 426$ (attributed as blue- or short-wavelength–sensitive pigment), ≈ 530 (green- or mid-wavelength–sensitive pigment), and ≈ 558 nm (red- or long-wavelength–sensitive pigment), respectively (Merbs and Nathans 1992). For the red and green pigments, two variant forms linked to alanine/serine substitutions at amino acid residue 180 are found in different proportions in Caucasians.

The distribution of photoreceptors in the human retina display an almost radial symmetry, with the number of rods ($\approx 100 \times 10^6$) greatly exceeding the total number of cones ($\approx 6 \times 10^6$). In cross section, the distribution of cones shows a bell-shaped profile, with a peak in the central 1° of the retina, the *fovea centralis*. This region lacks rod as well as blue-cone photoreceptors and represents the area of highest visual acuity. The density

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of rods increases with distance from the central retina and reaches its maximum at 18° eccentricity. Blue cones, which constitute only ~10% of all cones, are most frequent in the pericentral retina.

The 19th-century physiologists Maxwell, von Helmholtz, and Young discovered that any light stimulus in the visible spectrum could be matched by the mixture of three spectrally pure lights of particular wavelengths, and thus they established the hypothesis of trichromatic vision in humans. The sensitivity curves deduced or obtained from these early theoretical or psychophysical studies fit remarkably well with the absorption spectrum of the human cone pigments.

Cone-Pigment Genes and the Evolution of Human Color Vision

Nathans and et al. (1986) cloned and characterized the genes that encode for the human cone pigments. They showed that the red and green pigments are highly conserved, with 96% identity at the amino-acid level, but that they are only distantly related (40%–44% identity) to either the blue pigment or rhodopsin. Whereas the genes that encode for rhodopsin and the blue-cone pigment are located on autosomal segments, 7q31-q32 and 3q21-q24, respectively, the genes for the red- and green-cone pigments colocalize in a head-to-tail tandem array on human chromosome Xq28 (Vollrath et al. 1988). Unequal homologous recombination and gene conversion events between the adjacent red- and green-pigment genes have probably contributed to the high sequence conservation between both genes. The divergence of red- and green-pigment genes in primates obviously happened very recently in evolution (~30 million years ago) and is probably the result of the duplication of an ancestral mid-/long-wavelength (MW/LW)–sensitive pigment gene. Catarrhine primates, which include Old World monkeys, great apes, and humans, possess two spectrally different MW/LW cone pigment genes, whereas most New World monkeys have developed a single-gene allelic system to achieve trichromatic vision. Their single MW/LW cone opsin gene on the X chromosome is polymorphic and encodes pigments with differences in peak absorbance of $\leqslant 20$ nm. Therefore, female monkeys with alleles that encode spectrally different MW/LW cone pigments have trichromatic vision, whereas males are obligate dichromats (Jacobs 1996). On the basis of sequence comparisons of the MW/LW cone pigment genes, it has been suggested that both trichromatic systems in primates have evolved independently and represent an example of convergent evolution to adapt to the need to detect colored fruits against the background of dappled foliage (Shyue et al. 1995).

Structural Variability of the Human Red- and Green-Pigment Gene Cluster

The copy number of red/green-pigment genes varies considerably among individuals, which gives rise to a complex set of pigment gene arrays. Each repeat unit consists of an ~15-kb pigment gene segment and ~24 kb of intergenic sequence (Vollrath et al. 1988). The high conservation between red- and green-pigment genes, which includes intron sequences and intergenic repeat sequences, predisposes this arrangement to unequal homologous recombination, which results in the deletion/insertion of repeat units. Intragenic recombination between red- and green-cone pigment genes results in hybrid genes, which are found frequently in subjects with color-vision abnormalities but also in some color-normal individuals (for a review, see Nathans et al. 1992; Deeb and Motulsky 1996). As originally proposed by Nathans and colleagues, most researchers favor a model with a single red-pigment and up to five downstream green-pigment genes, with a mean range of 2.78–3.15 pigment gene copies per X chromosome (Nathans et al. 1986; Drummond-Borg et al. 1989; Macke and Nathans 1997; Yamaguchi et al. 1997). Neitz and Neitz (1995), however, calculated a mean number of 4.3 copies and proposed that some males carry up to four red-pigment genes and up to nine pigment gene copies in total. Although the additional red pigments predicted in this study must be assigned structurally as 5′ green–3′ red hybrid genes, their presence in ~50% of color-normal subjects is at odds with the low proportion (3%–8%) of hybrid genes reported in other studies. Given the near equal frequency of alleles that encode for the two spectrally different red-pigment variants ($180^{Ser}/180^{Ala}$) in the Caucasian population, the presence of multiple red-pigment genes raises fundamental questions about the generality of von Helmholtz and Young's theory of trichromatic vision. However, comparative analyses using the same set of normal subjects have shown that molecular techniques commonly used to calculate copy number and red-to-green-pigment gene ratios are of only limited accuracy. By calibrating the copy number of the red/green-pigment gene array by means of pulsed-field electrophoresis and by visualizing the array directly with the fiber FISH technique, we found at most six pigment genes, with a mean copy number of 3.31 and a proportion of 8.6% normal subjects with additional hybrid genes (S. Wolf, H. Schmidt, L. T. Sharpe, and B. Wissinger, unpublished data).

Selective Expression of Red- and Green-Pigment Genes

The presence of hybrid genes in addition to the normal red- and green-pigment genes suggests that some males

might express more than three different cone pigments. However, RT-PCR analyses of retinal mRNA from human donors with multiple green-pigment genes have shown that only one of these genes is expressed at a detectable level (Winderickx et al. 1992). Moreover, it has been shown that, in retinae of unselected men who carry a 5′ green–3′ red hybrid gene in addition to the normal red- and green-pigment genes, only two types of transcripts can be detected. These represent the normal red as well as either the normal green or the hybrid-pigment gene sequences (Yamaguchi et al. 1997). It has thus been reasoned that expression of the human red/green-pigment gene cluster occurs mainly from the first two genes in the array, independent of the pigments actually encoded at these two loci. Red/green-pigment ratios, quantitated at the mRNA level from the retinae of normal males, indicate that the red-pigment gene is always expressed in excess of the green pigment, irrespective of the number of pigment genes in the array (Yamaguchi et al. 1997).

Expression of pigment genes is under the control of a so-called locus control region (LCR), a sequence element 3.1–3.7 kb upstream of the proximal pigment gene. This LCR is essential to drive expression of a *lacZ* transgene in mouse cone photoreceptors (Wang et al. 1992). By analogy with the well-studied LCR in the β-globin cluster, it is proposed that the X-linked pigment gene LCR interacts with downstream promoters of the red- and green-pigment genes and favors the expression of proximal copies of these genes. Since it is generally believed that only one pigment is expressed in a given photoreceptor, it follows that this LCR-promoter interaction, once established, is maintained during the life of the cell. However, neither the molecular mechanisms that underlie this model nor the processes that determine the fate of a photoreceptor progenitor in becoming a blue, red, or green cone have yet been unraveled.

Color-Vision Anomalies and Deficiencies Caused by Pigment Gene Defects

Color-vision deficiencies (table 1) are generally congenital and nonprogressive conditions, and their existence and hereditary character were described as early as the 18th century. One of the first reports was that of John Dalton, who recognized himself and two of his brothers as "red-green blind" subjects. The various forms of red/green dyschromatopsia are often referred to as Daltonism, in his honor. In 1876, Horner proposed that inheritance of this condition was sex linked, and the linkage of hemophilia and color blindness, shown by Bell and Haldane in 1937, was among the first of such reports in human genetics (François 1961).

The common X-linked forms of color-vision anomalies and defects are often referred to as red-green color blindness, a misleading phrase because the phenotype is not color blindness but rather a reduced ability to match or discriminate colors in the mid- to long-wavelength spectrum. On the basis of psychophysical tests, subjects in this group can be classified as true dichromats, who are either protanope ("red blind") or deuteranope ("green blind"), or as abnormal trichromats, who are either protanomalous ("red weak") or deuteranomalous ("green weak"). Between 3% and 8% of the male population is affected, dependent on the ethnic group studied. Deuteranomaly accounts for ~50% of these cases, and the other three types of deficiencies contribute in nearly equal proportion to the remainder (François 1961; Jäger 1971).

These traits are linked to the red/green-pigment gene cluster on chromosome Xq28 and are mainly caused by structural rearrangements, which probably result from unequal homologous recombinations between red- and green-pigment genes. Detailed descriptions of the molecular basis of this common color-vision deficiency have been published (Nathans et al. 1992; Deeb and Motulsky 1996). In general, protanopia and protanomaly are associated with the presence of a 5′ red–3′ green-hybrid gene at the first position of the array. Given the small number of amino acid differences that account for most of the spectral absorbance differences between red and green pigments ($180^{Ser/Ala}$, $277^{Tyr/Phe}$, and $285^{Thr/Ala}$), the original allelic composition and the location of the point of fusion largely determine the functional character of the hybrid gene and therefore the type of color-vision deficiency. In contrast, deuteranope and deuteranomalous subjects carry a single normal red-pigment gene, which either represents the sole pigment gene or occurs with a 5′ green–3′ red hybrid gene in the second position of the array. The phenotypic severity is again, in these cases, determined by the composition of this hybrid gene. Whereas these structural rearrangements are fairly common, only one missense mutation (Cys203Arg) has been described. This mutation was present in all downstream copies of the green-pigment gene in a deuteranomalous subject.

The term "tritanopia" indicates the weak or absent discrimination of short-wavelength blue-yellow stimuli. Inherited tritanopia is unique among color-vision deficiencies because of its autosomal dominant transmission. With only a few surveys available, the frequency of tritanopia has been variously estimated to be 1:500–1:65,000. Analysis of the blue-cone pigment in a small group of families with tritanopia has revealed heterozygous missense mutations, Gly79Arg, Ser214Pro, and Pro264Ser, which cosegregate with the disease in the analyzed families. Incomplete penetrance of the Gly79Arg mutation has been observed (Weitz et al. 1992). The dominant effect of mutations in the blue-cone pigment is reminiscent of the rhodopsin gene mu-

Table 1

Human Hereditary Color-Vision Deficiencies (Caucasian)

Disorder	Frequency (males/females)	Inheritance	Gene Defect[a]
Red/green color–vision deficiencies:			
Protanopia	~1:100/1:10,000	XLR	RCP
Protanomaly	~1:100/1:10,000	XLR	RCP
Deuteropia	~1:100/1:10,000	XLR	GCP
Deuteranomaly	~1:25/1:625	XLR	GCP
BCM	~1:100,000 (males)	XLR	RCP/GCP
Tritanopia	1:500–1:65,000	AD	BCP
Total color blindness	1:20,000–1:50,000	AR	CNGA3

[a] RCP = red-cone pigment, GCP = green-cone pigment, and BCP = blue-cone pigment.

tations in retinitis pigmentosa, which are almost always dominant.

Blue cone monochromacy (BCM) is a rare X-linked recessive condition that results from the lack of both red and green cone pigments. Its frequency has been estimated at 1:100,000. BCM individuals share many clinical features of classical color blindness: very poor or absent color discrimination, photophobia, severely reduced visual acuity, and pendular nystagmus. However, residual blue-cone photoreceptor function can be detected in psychophysical threshold tests and in electroretinographic recordings. Furthermore, although only a single type of cone pigment is present in BCM, it has been shown that some color matches in the region between 400 and 500 nm are possible under dim light conditions, when both the rod and blue-cone systems are mediating vision.

Multiple classes of mutations can lead to BCM. A large proportion of BCM patients carry only a single red or 5′ red–3′ green hybrid pigment gene that harbors a Cys203Arg missense mutation, although BCM individuals are also observed who carry up to three pigment genes, each of which contains this lesion. Other BCM patients show various sized deletions (0.6–55 kb) of the proximal part of the red/green cone pigment gene cluster. In all such cases, the deletion affects the LCR, consistent with its presumed essential role in activating the expression of adjacent cone-pigment genes (Nathans et al. 1993).

Genetic Basis of Complete Color Blindness

Total color blindness, also referred to as complete achromatopsia or rod monochromacy, is the severest form of color-vision deficiency, with a complete lack of color discrimination. Because of their drastically reduced visual acuity and pronounced photophobia, such individuals are severely visually handicapped, and some may be considered legally blind. Electroretinographic recordings confirm a complete lack of cone photoreceptor responsiveness (for a review, see Hess et al. 1990). Inherited as an autosomal recessive trait, total color blindness has been estimated to affect 1:20,000–1:50,000 (François 1961; Jäger 1972). In some isolated populations, this condition seems to be much more frequent, as recounted in Oliver Sacks' popular book *The Island of the Colorblind*. Sacks describes the people of the remote Pacific island of Pingelap, of whom ~10% suffer from color blindness (Sacks 1997). This high incidence is attributed to genetic drift; most of the inhabitants are said to have been killed in a devastating typhoon, and the small group of survivors who repopulated the island no doubt included a carrier of the mutant gene for color blindness.

Molecular analysis of total color blindness started incidentally with the description of maternal isodisomy of chromosome 14 in a single affected person (Pentao et al. 1992). Subsequent analysis of a large inbred Iranian Jewish family excluded chromosome 14 but showed significant linkage with markers in the centromeric region of chromosome 2 (Arbour et al. 1997), a result that was confirmed in families from Germany, Norway, and the United States. Mapping of recombination breakpoints and homozygosity intervals enabled a refinement of the locus to a 3-cM interval on 2q11 that contained the candidate gene *CNGA3*, which encodes the cone photoreceptor α-subunit of the cGMP-gated cation channel (Wissinger et al., 1998). Subsequent mutational analysis of *CNGA3* revealed the missense mutations that cosegregate with color blindness in the families analyzed (Kohl et al. 1998).

Although blue and red/green cones evolved early in vertebrate phylogeny and differ markedly in their physiological properties, the complete lack of cone function in patients with *CNGA3* mutations implies that all three types of cone photoreceptors in the human retina require this gene. In contrast, the α-subunit of the cGMP-gated channel that is expressed in rod photoreceptors is encoded by a different gene, *CNGA1*. It is of interest that

some patients with autosomal recessive retinitis pigmentosa carry mutations in *CNGA1* (Dryja et al. 1995). This progressive type of retinal dystrophy, caused by mutation in the rod channel, contrasts with the congenital and static phenotype of people with total color blindness.

Rod and cone photoreceptor cGMP-gated cation channels belong to a small family of cyclic nucleotide-gated (CNG) channels, which act not only in vision but also in some other sensory systems (for a review, see Zagotta and Siegelbaum 1996). The cAMP-gated channel expressed in the sensory neurons of the vertebrate olfactory epithelium serves as the main mediator of responses to odorants. Knockout mice lacking these olfactory cAMP-gated channels show anosmia, the complete lack of odor perception (Brunet et al. 1996). In addition, CNG channels appear to participate in thermo- and chemosensation in *Caenorhabditis elegans* (Komatsu et al. 1996), and they may also act in various nonneural cells. For example, one CNG channel seems to be responsible for the influx of Ca^{2+} ions into sperm that accompanies chemotaxis. Although obviously monophyletic in origin, CNG channels have adapted to carry out conserved physiological functions in a number of different cell types as part of different sensory signaling pathways.

Mutations in the *CNGA3* gene in color-blind subjects affect amino acid positions of high evolutionary conservation and cluster in functionally important domains of the protein, particularly in the cGMP-binding domain and the transmembrane domain S4, which contains a putative voltage sensor motif similar to those found in voltage-gated K^+ channels. It may be argued that mutations in the cGMP-binding domain might decrease the actual affinity for cGMP and thus keep the channel in a closed state, a situation analogous to continuous light stimulation. However, permanent closure of these channels would obviously alter the resting potential of the cell and would most likely abolish photoreceptor excitability.

The question remains whether total color blindness is necessarily associated with mutations in *CNGA3*. Exclusion of linkage to 2q11 in some families does indeed indicate genetic heterogeneity of this condition (S. Kohl and B. Wissinger, unpublished data). By analogy with the separation of white light into different colors by use of a glass prism, it seems that we may have encountered only a part of the genetic spectrum of color blindness.

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