G PROTEIN-COUPLED RECEPTORS

Hue and the heptahelicals

John Mollon

MUCH of the intercourse between our nerve cells, and much of our interaction with the outside world, depends on members of the same superfamily of protein molecules[1]. These molecules, embedded in cell membranes, are characterized by seven helices that span the membrane, and their action within the cell is achieved by G proteins. Commonly (and clumsily) known as G protein-coupled receptors, they might be better named heptahelical receptors. Already the genes for several hundred heptahelicals have been sequenced; R. J. Lefkowitz provided a helpful discussion of them and summarized the known ligands in News and Views last month[2].

The opsins (the protein parts of the photosensitive pigments of the retina) are particularly well-studied members of the heptahelical family. Because they exhibit a rich polymorphism in humans and primates, and because a number of pathological mutations have been identified (in cases of retinitis pigmentosa[3,4]), they offer useful models to those who study their more distant relatives[5]. Now, in *Science*[6], Neitz *et al.* put forward a theory of how the middle-wave and long-wave cone pigments of human vision come to differ in their spectral absorbances (that is, in the way their sensitivity varies with the wavelength of the incident light).

Since 1986, when Nathans and his colleagues published sequences for the human cone pigments, it has been known that the long-wave and middle-wave opsins differ only in 15 amino acids and only seven of these are non-homologous substitutions lying within the transmembrane regions where they are likely to affect the absorbance properties of the molecule[7]. To home in on the amino-acid sites that are critical, Neitz *et al.* have now exploited the striking polymorphism of colour vision found in many New World monkeys, such as marmosets, tamarins and squirrel monkeys[8]. Within a given species, the hue discrimination of the male monkeys resembles that of dichromatic men (that is, men who are colour blind in the sense that they can match all colours with two variables), whereas most of the female monkeys are trichromatic, enjoying good discrimination within the red–green range. Moreover, within each species, there appear to be at least three kinds of dichromat and three kinds of trichromat, differing in their exact spectral sensitivity.

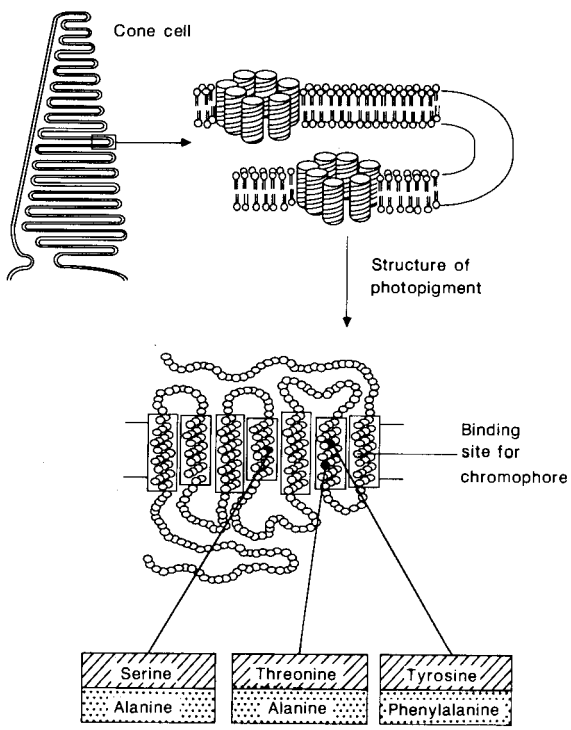
A decade ago, Bowmaker, Jacobs and I showed that these behavioural variations are associated with variations in the retinal photopigments[9]. That work led to a genetic model which supposes that New World monkeys, unlike ourselves, have only a single locus for specifying a pigment with peak sensitivity in the red–green range of the spectrum[10]. This locus, like the loci for the human long- and middle-wave pigments, is thought to be on the X chromosome. Three alleles can occur at the monkey's single locus, so giving the three kinds of male dichromat. The three alleles occur with similar frequency, so that many females will be heterozygous, inheriting different versions of the gene on their two X chromosomes. Owing to X-chromosome inactivation, the heterozygous female will express only one allele in any given cell; and apparently her visual system can exploit the presence of two subtypes of cone. As all the monkeys also have a short-wave pigment, the heterozygote becomes trichromatic.

These polymorphisms offer an attractive way of discovering the molecular differences that give a photopigment its spectral sensitivity. Neitz *et al.* have directly sequenced the genes for the 562-nm, 556-nm and 541-nm pigments that occur individually in different male tamarins, and the 561-nm, 547-nm and 532-nm pigments that occur in male squirrel monkeys. They find pairs of pigments that differ at only one of the seven sites considered relevant in the case of human long-wave and middle-wave pigments. Thus the 556-nm tamarin pigment differs from the 562-nm tamarin pigment only by the substitution of alanine for serine at position 180; the 541-nm tamarin pigment differs from the 556-nm tamarin pigment only by the substitution of alanine for tyrosine at position 285; and the 547-nm squirrel monkey pigment differs from the 556-nm tamarin pigment only by the substitution of phenylalanine for tyrosine at position 277 (see figure). In each case a non-polar amino acid replaces a hydroxyl-bearing residue.

Neitz *et al.* are led to the bewitchingly simple theory that the three sites (180, 277 and 285) account for all the variation in the spectral sensitivity of primate cone pigments in the range 530 to 565 nm and that the three substitutions are linearly additive in the number of nanometres by which they shift the peak sensitivity of the photopigment. The best estimate for the shift produced by the substitution at site 180 is 5.3 nm; and for the substitutions at sites 277 and 285 the best estimates are 9.5 and 15.5 nm respectively. These three substitutions sum to give the approximately 30 nm difference between the human long- and middle-wave pigments.

How secure is this additive theory? Neitz *et al.* show that it accurately predicts paired comparisons among all eight of the pigments considered and they also show that the substitutions observed at other sites cannot predict such additive shifts. But they restrict themselves to the subset of alternative theories in which individual substitutions act alone in an additive way; they do not consider more complicated theories in which additional, facilitating, substitutions are required to produce the full 30-nm shift between the human long-wave and middle-wave pigments. The theory can be tested in two ways: one is to exploit nature's own experiments and to sequence more opsin genes; the other is to change individual amino acids by site-directed mutagenesis and measure the expressed pigment — as has already been done with rhodopsin[11,12].

Neitz *et al.* end their paper by suggesting that two forms of the long-wave opsin are present in the human population (a possibility earlier suspected from microspectrophotometric results[13]): they propose that the two pigments differ according to whether alanine or serine is present at site 180 and



Visual transduction depends on a G protein-coupled molecule embedded in the multiply infolded membrane of the outer segment of a cone cell. The opsin (the protein part of the molecule) is bound to the chromophore, 11-*cis*-retinal, at the site indicated in the lower part of the diagram (seventh helix). The additive theory of Neitz *et al.* proposes that just three amino acids (filled circles in the cartoon of the molecule) determine the spectral difference between the human long- and middle-wave pigments. The pairs of alternative amino acids are shown in boxes at the bottom of the diagram and in each case it is the upper of the two that produces a red shift in the absorbance of the molecule. (Figure by P. Jeffs.)

thus differ by 5 to 6 nm in the wavelength of maximum absorbance. Earlier, in 1986, Neitz and Jacobs suggested that men fall into two clear groups on the basis of their subjective colour matches[14]. The finding has been controversial[15], and at the recent meeting of the Association for Research in Vision and Ophthalmology, J. Neitz implicitly withdrew the claim[16]. What he and his colleagues now suggest is that a subset of men carry copies of two forms of the long-wave gene, expressing them in different cones. Such 'pseudo-heterozygotes' may reveal themselves by changes in their colour matches after selective colour adaptation. It was in these pages 110 years ago that Lord Rayleigh suggested that different observers may live in slightly different perceptual worlds[17], and some of this variance can now be traced to variations in heptahelical receptors. Perhaps polymorphisms of other heptahelicals account for some of the variation that is observed in people's mental worlds. □

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