Perspective

# The Molecular Biology of Color Vision

DAVID BOTSTEIN

THERE ARE PROBABLY AS MANY REASONS TO DO SCIENCE AS there are scientists. Too often, the esthetic excitement of science is sacrificed to its undoubted utilitarian value, a trend that seems to be intensifying. Still, many scientists remain who appreciate the esthetics of the process from discovery to understanding, and for them a treat is in store. The elements of beautiful science are familiar: first the confrontation of the human mind with a natural phenomenon, then its investigation through observations and experiments, the continuing proposal of theories, the testing of predictions, and finally, in best case, the convincing demonstration of the validity of one of the theories through confirmation of its specific predictions. The process can take only a few years and involve only a few scientists or it can span centuries and involve many. The practical consequence may be revolutionary and change the course of history (for example, special relativity) or it may have little or no use. In either case, a full scientific story, especially one that has been unfolding over historic times, can be a lovely thing, like a classical symphony or a gothic cathedral.

The present issue of *Science* contains the satisfying resolution of such a story, one that began with the earliest philosophers and scientists, engaged the interest of many of the greatest names of science, some of whom (including Newton, Dalton, Helmholtz, Maxwell, and Schrödinger) contributed materially to the theories now confirmed. The subject is the mechanism of color vision, specifically the number and relationship of the primary receivers of color information. The substantially correct theory was first proposed by Young in 1802 (*1*), based on the physical analysis of the nature of color in light by Newton. The observations that require explanation include the nature of the defects in color-blind individuals. The relevance of the details of color blindness and the important constraints these place on theories of color vision had first been recognized by Dalton (*2*), who noted in 1798 that whereas others saw a universe of color that could be filled completely only by mixing three primary colors, he himself saw a universe satisfied by mixtures of two primary colors, yellow and blue. Dalton, the father of the atomic theory of matter, thus correctly diagnosed himself as a dichromat, while introducing this basically correct view of the most common form of color blindness. Maxwell extended and generalized mathematically this view of dichromatism, and the heuristic device he used is still standard in the field and called the Maxwell triangle.

The new insight into color vision has come through the application of the modern methods of molecular genetics. The work illuminates not only the physiology of color vision, but also basic mechanisms of evolution. It has been thought for some time that a major theme of evolution is duplication followed by divergence. The discovery that many genes are split into discontinuous coding segments led to the idea that rearrangements among such duplicated genes might result in the acquisition of useful novel functions. The new results on color vision provide a fine example of duplication, divergence, and, probably, "exon shuffling" as it is actually happening in the genome of our species.

The two articles in this issue of *Science* are by Jeremy Nathans and co-workers at Stanford, who describe the isolation of the genes that specify the protein moieties ("opsins") of the three different color-sensitive pigments in the human eye (*3, 4*). Nathans *et al.* used DNA probes from these genes to analyze the defects in the DNA of color-blind individuals by hybridization to Southern blots. They confirm, in this way, that there is indeed a gene that specifies opsins for pigments that recognize primarily red, others that recognize primarily green, and yet others that see primarily blue. Two of these (specifying red-sensitive and green-sensitive opsins) comprise a family of repeated genes on the human X chromosome.

The molecular genetics is interesting in itself. The red, green, and blue pigment genes were molecularly cloned as DNA molecules by application of evolutionary principles. The bovine rhodopsin gene was used to identify, by DNA hybridization, conserved sequences in the human. The paradigm of duplication and divergence predicted that all the pigment proteins would be related: bovine to human, and the different color pigment proteins to rhodopsin and to each other. This strategy succeeded, and the gene for human rhodopsin was readily found by homology with its bovine counterpart. The color opsins turned out to be more distantly related but, by reducing the "stringency" of the hybridization, three different color pigment opsin genes were obtained as well. While this is by no means the first (or even most spectacular) application of the principle of conservation in this way, it is nicely illustrative of the way in which evolutionary predictions can be used to practical advantage.

The most elegant result of Nathans and colleagues is the identification of the red and green pigments with their corresponding DNA clones. It is a fine example of the way that much can be learned about the function of genes only by using DNA clones to study genetic variants. Three different opsin-encoding sequences were cloned; some of the genes were present in a variable number of copies in different individuals. It remained to determine functionally what these genes did for color vision. This was accomplished first by using the fact that red-green color blindness (protanopia or deuteranopia) is an X-linked trait in humans. It is much more prevalent in men (who have one copy of the X chromosome) than in women who, having two X chromosomes, are usually heterozygous for the recessive defect and do not show the symptoms although they pass the defect on to half their sons who, having only the one X chromosome, are color blind. Nathans *et al.* located two of the three opsin gene classes to the X chromosome. These therefore became candidates for the red and green functions while the third class by difference is inferred to be a candidate for the blue opsin gene. Nathans *et al.* then discovered that the green and red genes are in a tandem array, all oriented with the tail of one gene near the head of the next. When the DNA of protanopes (hypothesized by the Young-Helmholtz theory to be missing the ability to see red) was analyzed, loss or alteration of one of the genes (the first in the array) was observed. This result allowed the inference that this gene specifies the red opsin. Consistently, when deuteranopes (hypothesized to be missing the ability to see green) were examined, the other X-linked genes (the number is variable) were missing or altered. Thus the combination of molecular and genetic analysis allowed the unambiguous identification of gene (as DNA) with function—the precise alteration of color vision.

The observation that the genes are in a tandem array, plus the

David Botstein is a professor of genetics in the Department of Biology at Massachusetts Institute of Technology, Cambridge, MA 02139.

variable number, allowed Nathans *et al.* to explain how the color blindness defect might be generated at the DNA level. The red and green genes are very similar in DNA sequence, and homologous recombination between them could occur. Recombination between different members of the same tandem array (often called "unequal crossing-over") has been observed in many organisms (as early as the second decade of this century in *Drosophila*) and is thought to account for much evolution in man, beginning with observation that first indicated the existence of tandem arrays of gene families in humans, the delta-beta hybrid hemoglobin (hemoglobin Lepore). If such unequal crossing-over were to occur in the X-linked opsin family, it would sometimes result in the loss of the red gene and sometimes the loss of all the functional green genes. Amplification of the number of genes and the production of red-green hybrid genes are also possible consequences of unequal crossing-over. As might be expected, the variation in the number of green opsin gene copies has effects on the details of color vision.

The tandem array also tempts one to speculate about the evolutionary history of color vision in our species. It seems plausible that the present trichromatic system evolved from a dichromatic system: either red or green (or a common ancestor) on the X chromosome and blue on another chromosome. The ancestral red-green gene then duplicated and diverged, resulting in the present arrangement. The high degree of homology between the red and green genes and the continuing variation in number (presumably by unequal crossing-over) support such an explanation. The observation that New World monkeys have only a single pigment encoded on the X chromosome lends additional strong support for this view. These results also illustrate how relatively unstable duplication-divergence is in evolutionary time, at least until the degree of divergence (or translocation to another chromosome) reduce the ease of reversal of the duplication that made two genes from one.

Icing for this cake comes from the analysis by Nathans *et al.* of the DNA of men classified as "anomalous trichromats": that is, men whose color universe requires three primary colors, but who nevertheless see differently from normal people. The Young-Helmholtz theory postulates, in these cases, red- or green-sensitive cones with abnormal pigment function. Nathans *et al.* examined many such subjects and found in each case the presence of hybrid genes that might account for the anomalous function. All that is required is to postulate that the hybrid genes can produce hybrid opsins that localize into the correct cone, but that nevertheless have an altered action spectrum.

What is particularly engaging about this kind of result, of course, is the possibility that such a process might represent an example of ongoing further evolution of the visual system. One could easily see how the extra copies of the green opsin gene already present in most people could, by further unequal crossing-over or mutation, evolve into a third X-linked pigment with a new spectrum that could extend the visual abilities of those who inherit it. If, in the future, selective pressures are applied for better color discrimination, the anomalous trichromats may already have genes allowing increased fitness and survival.

There is much to be learned still about the physiology and molecular genetics of color vision. The major themes are, however, now clear. Through the application of modern recombinant DNA techniques and the analysis of genetic variants, a problem as old as the human effort to understand the real world has been brought to a higher, and most satisfactory, level of understanding. From the point of view of the Young-Helmholtz theory, dramatic confirmation is at hand; from the point of view of evolution of gene families, a nice illustration has been found.

REFERENCES


1. T. Young, *Phil. Trans. R. Soc.* **12** (1802).
2. J. Dalton, "Extraordinary facts relating to the vision of colours," *Mem. Lit. Philos. Soc. Manchester* **5**, 28 (1798).
3. J. Nathans, D. Thomas, D. S. Hogness, *Science* **232**, 193 (1986).
4. J. Nathans, T. P. Piantanida, R. L. Eddy, T. B. Shows, D. S. Hogness, *ibid.*, p. 203.