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Jeremy Nathans' Lecture Part 2:
Human Color Vision

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1. Keywords and Terms

trichromatic color vision, refraction of light, color mixing, cone pigments, spectral sensitivity, visual pigment genes, gene duplication, color blindness, unequal recombination, polymorphic variation

2. Lecture Notes

Isaac Newton's prism experiments: sunlight is made up a continuous series of lights that differ in their angles of refraction when passed through a prism, the different lights

appear as different colors, and various combinations of these lights can create the color sensation of a physically different light.

Thomas Young's hypothesis that human trichromatic color vision was based on three types of light sensors within the retina, with distinctive absorbance curves that are broad and substantially overlapping.

The absorbance spectra of the human visual pigments and the resulting wavelength discrimination curve measured psychophysically.

James Clerk Maxwells' conceptualization of color space as a three dimensional Cartesian coordinate system

The chemical basis for spectral tuning among visual pigments that use the same 11-cis retinal chromophore but differ in their absorbance spectra. The key determinant of the position of the absorbance curve is the degree of pi-electron delocalization: the more delocalization, the greater the red-shift.

The amino acid sequences of human visual pigments reveal a high degree of similarity between M and L pigment genes.

An analysis of the genes coding for the human visual pigments shows that the L and M pigment genes reside in a head-to-tail tandem array on the X-chromosome. This arrangement is the result of a relatively recent gene duplication. The S pigment gene and the rhodopsin gene are on autosomes.

The common variations in human color vision: dichromacy (two rather than three types of cones) and anomalous trichromacy (one of the three cone types has a shifted absorbance spectrum). The vast majority of individuals with a color vision variation are males and they have the variation within either the M or L pigments – a simple consequence of the X-chromosome location of these two genes.

Arrangement and rearrangement of L and M pigment genes as a consequence of frequent unequal homologous recombination, either between the genes (intergenic recombination) or within the genes (intragenic recombination). The result: variability in

the number of genes in the array, and the common finding of hybrid genes (one part from an L pigment gene and one part from an M pigment gene).

A common single amino acid polymorphism in the human gene pool (alanine vs. serine at position 180 in the L pigment gene) gives rise to a several nanometer shift in spectral sensitivity, dividing the population of human trichromats into different perceptual groups.

John Dalton's many contributions to the study of variant color vision: his astute description of his own dichromacy, his instructions that his eyes be examined post-mortem for signs of a pre-retinal discoloration (which was not found), and finally, 150 years later, DNA analysis showing that he was missing the M pigment gene.

3. Recommended Reading

1. Hunt, D.M., Dulai, K.S., Bowmaker, J.K., and Mollon, J.D. (1995) The chemistry of John Dalton's color blindness. Science 267: 984-988.
2. Lennie, P. (2001) Color Vision (chapter 29) in Principles of Neural Science (fourth edition). Kandel, E.R., Schwartz, J.H., Jessell, T.M., editors. New York: Elsevier.
3. Mollon, J.D. (1989) "Tho' she kneel'd in that place where they grew…" The uses and origins of primate color vision. Journal of Experimental Biology 146: 21-38.
4. Nathans, J. (1989) The Genes for Color Vision. Scientific American (February): 42-49.

4. Review Questions

1. What happens to sunlight when it is passed through a glass prism?
2. Describe the absorbance spectra of the three human cone pigments.
3. How does pi-electron delocalization affect the absorbance spectrum of the visual pigment chromophore 11-cis retinal?

4. Among the three human cone pigments, which two are most similar in amino acid sequence?

5. What characteristics of the human M and L pigment genes leads to the high frequency of variation in their structure?

6. Why are variations in human color vision more common among men than women?

7. Among individuals who are missing either the M or L pigment, which wavelengths of light are difficult to discriminate?

8. What is the single most common genetically-determined variation in human color vision?

5. Answers to Review Questions

1. It is split into its component rays that differ in wavelength and angle of refraction. Longer wavelength light bends least and shorter wavelength light bends most. The result is a spectrum of colors proceeding from red (bent least) to orange, yellow, green, blue, and violet (bent most).

2. Each absorbance spectrum has a broad bell shape when plotted along the wavelength axis, and the spectra show substantial overlap. The curves peak at 440 nm (the short-wave or S pigment), 530 nm (the medium wave or M pigment), and 560 nm (the long wave or L pigment).

3. For molecules with alternating single and double bonds, such as 11-cis retinal, greater pi-electron delocalization leads to a smaller energy gap between the ground state and the photo-excited state, and therefore a shift of the absorbance to longer wavelength.

4. The M and L pigments.

5. The M and L pigment genes are nearly identical in sequence, and they are located adjacent to one another in a head-to-tail tandem array. These two factors predispose these genes to unequal homologous recombination.

6. The M and L pigment genes reside on the X-chromosome. Men have a single X-chromosome, whereas women have two X-chromosomes. Therefore, men who inherit an alteration in the structure of the L and/or M pigment genes within their

single X-chromosome will have altered color vision. In women, variation in the M and/or L pigment genes cause variant color vision only in those relatively unusual individuals who have inherited alterations in these genes on both of their X-chromosomes. Variations in the S pigment gene are relatively rare and affect men and women with equal frequency.

7. This pair of pigments absorbs light at the longer wavelength end of the spectrum, whereas the S pigment absorbs light at the shorter wavelength end of the spectrum. The absence of either the M or L pigment impairs discrimination of longer wavelengths, corresponding to the color sensations that we call red, orange, yellow, and green.

8. A single amino acid variation in the human L pigment gene (alanine or serine at position 180) causes a several nanometer shift in the absorbance spectrum of the L pigment.

6. Discussion Questions

1. What is the origin of a rainbow?

2. Consider the curve that describes the minimal wavelength difference that is required to discriminate a pair of spectrally pure lights as a function of their wavelengths. For trichromatic humans, this curve has a distinctive and complex shape, with minima at about 480 nm and 580 nm. How can we explain this distinctive shape?

3. Starting with a pair of M and L pigment genes in their normal head to tail tandem arrangement, with the L pigment gene 5' of the M pigment gene, draw the various products that arise from homologous intergenic and intragenic meiotic recombination and predict the color vision phenotypes that would be conferred on a male who inherited each of the resulting X-chromosomes. Note that the hybrid pigments that are produced from M/L or L/M hybrid genes (following intragenic recombination) invariably have absorbance spectra that are in the interval between the normal M and L pigments. As a more advanced exercise, try starting with X-chromosomes each of which carries one L pigment gene followed by two M pigment genes (the most common arrangement in the human gene pool).

4. Thus far, there are no reports of anomalous trichromats with a visual pigment that has a spectral sensitivity at longer wavelengths than the normal human L pigment. Is this surprising?

5. For humans, the visible region of the spectrum ranges from about 400 nm to about 700 nm. Is vision possible beyond this range?

7. Answers to Discussion Questions

1. A rainbow is seen when small, and therefore nearly spherical, water droplets are suspended in the atmosphere, and there is an unobstructed path between the sun, the water droplets, and the viewer. Under these conditions, the water droplets act like tiny prisms, although with a somewhat more complex light path than the path through the glass prisms that Isaac Newton used. In the case of the primary rainbow, the light path consists of the entry of a beam of light into the droplet at the edge of the sphere, its partial reflection from the back of the water droplet, and then its exit from the opposite side of the sphere. In going from air to water and then back again from water to air the light rays will be refracted in the way that they are refracted in going from air to glass and then glass back to air. When viewing conditions are especially good it is possible to see a double rainbow, in which case a second and fainter rainbow arises from a light path that involves two reflections within the water droplet. The order of colors in the secondary rainbow is reversed relative to the primary rainbow. These explanations were first formulated by Isaac Newton.

2. Chromatic discrimination is based on the differential activation of the three cones. In the human retina, the cone signals are processed by two circuits that act as differential analyzers: one circuit compares M and L cone signals, and the second compares the S cone signal to the sum of the M and L signals. To make a chromatic discrimination between two spectral stimuli the differential signal in one or both of these systems must be greater than some threshold value. If we look carefully at the absorbance spectra of the three cone pigments we can rationalize the discrimination curve. [To first approximation, the absorbance spectra of a cone pigment is equivalent to the sensitivity curves of the corresponding cone photoreceptor if we neglect some subtle optical effects related to the waveguiding properties of cones.]

For example, at 580 nm, a small change in wavelength leads to a relatively large change in photon capture by the M pigment but a much smaller change in photon capture by the L pigment, because this wavelength is, respectively, on the steep descending long-wave limb of the M pigment absorbance curve but close to the peak of the L pigment. The S pigment and the S vs. (L+M) circuit are largely irrelevant in this instance because S-pigment absorbance at this wavelength is so small. The large differential signal in the L vs M circuit is sufficient to provide good chromatic discrimination at 580 nm.

At 450 nm, a small change in wavelength produces a relatively large a change in light absorbance by the L and M pigments, but both of these changes are similar as this wavelength is on the descending short-wave limb of the L and M absorbance curves. Therefore the differential signal in the L vs M circuit is small. At this wavelength, the S-pigment captures light efficiently, but because this wavelength is near the peak of the S-pigment absorbance curve, the S-pigment signal changes minimally with wavelength. Therefore, the differential signal in the S vs (L+M) circuit is also small. The result is poor chromatic discrimination 450 nm.

3. Images of the various homologous recombination events and their products can be seen in lecture 2, with the M and L pigment genes represented by arrows. It is apparent that these homologous recombination events can change the number of genes (by either intergenic or intragenic recombination) and produce hybrid genes (by intragenic recombination). Note that these homologous recombination events cannot decrease the number of genes to fewer than one.

   In the simplest case, the recombination event (either intergenic or intragenic) reduces the gene array to a single gene. In that case, the male who inherits this X-chromosome will be a dichromat, if we assume that the S pigment gene is normal. Exactly which type of dichromacy the person will have depends on whether the remaining gene is an L pigment gene, or an L/M hybrid gene. In the former case, the person will be a “deuteranope” (the official name for someone who is missing the M pigment). In the latter case, the person will be a “protanope” (the official name for someone who is missing the L pigment) if the L/M hybrid resembles the M pigment in its absorbance properties, which is almost always the case since the two most important amino acid differences for determining the M vs. L spectral sensitivity are coded in exon 5 near the 3’ end of each gene.

   The situation becomes both more complicated and more interesting if the M and L pigment gene array has more than one gene. Recall that for M and L pigment gene arrays with three or more genes, only the 5’ most two genes are expressed –

presumably this reflects an effect of distance separating the locus control region 5' of the array and the individual gene promoters. Thus, the color vision phenotype of a male with three or more genes in the array is determined simply by the absorbance spectra of the first two genes in the array. For example, if the first two genes are a normal L pigment gene and a normal M pigment gene, then the individual will have normal trichromatic color vision, even if more distal genes include M/L or L/M hybrids with anomalous absorbance spectra. As a second example, if the first two genes are an L pigment gene and M/L hybrid gene, and if the hybrid has an absorbance spectrum that is close to but not identical to the absorbance spectrum of the L pigment, then the individual will be a anomalous trichromat (officially called a "deuteranomalous" trichromat, which means the M pigment has been replaced by a pigment with a spectral shift). Someone whose color vision depends on two pigments with absorbance spectra that are closer together in spectral sensitivity than the normal M and L pigments will experience a reduced differential signal from the corresponding pair of cones, and, as a result, will have diminished chromatic discrimination in the red/orange/yellow/green (i.e. longer wavelength) region of the visible spectrum. Among Africans and Asians about 4% of X-chromosomes carry a hybrid pigment and confer anomalous trichromacy, and among Caucasians the frequency is about 8%. These are among the most common genetic variations in our species.

4. Even before the M and L pigment genes were isolated, it was inferred from color matching tests with different mixtures of spectrally pure lights that the anomalous pigments responsible for anomalous trichromacy had absorbance spectra in the interval between the normal M and L pigments. We now know that this observation reflects the fact that anomalous trichromacy results from the presence of hybrid pigments, encoded by M/L or L/M hybrid genes, and that the hybrids have absorbance properties that are intermediate between the two parental pigments. The fact that amino acid substitution mutations are far less common than unequal homologous recombination in this genetic system probably accounts for the failure, thus far, to identify individuals with mutant M or L pigments that lie outside of this interval. However, the possibility that mutant L or M pigments might have absorbance spectra outside of this interval is plausible because other species, such as birds and mice have visual pigments that are highly homologous to the human M and L pigments with absorbance spectra at wavelengths that are either shorter than the human M pigment (maximal absorbance at 530 nm) or longer than the human L pigment (maximal absorbance at 560 nm).

5. Yes. Many insects have excellent vision in the ultra-violet (UV; i.e. at wavelengths less than 400 nm), and not coincidentally many flowers have pigment combinations

that absorb or transmit light in the UV. Thus the insects that pollinate these flowers can see a set of distinctive colors that are invisible to us. However, seeing UV light has the problem that the higher energy of UV radiation can damage the retina. For long-lived animals like humans and other large mammals, avoiding the accumulation of UV damage over many years is probably more advantageous than seeing a few more colors. Thus, we come equipped with a very good short-wavelength cut-off filter to protect our retinas from UV light – the lens. Perhaps the accumulation of UV damage is less important for short-lived animals like insects.

As regards the detection of light on the long wavelength end of the spectrum, there is certainly room for a spectral shift of a few tens of nanometers toward the red end of the spectrum. However, for warm-blooded animals such as humans larger spectral shifts toward longer wavelengths will ultimately run up against a problem of signal-to-noise ratio because the heat from our bodies produces a constant background emission of infra-red (IR) radiation that would confound any IR light detector. Indeed, detecting IR radiation is the physical principle used for seeing humans and other warm objects with night vision goggles of the kind used in the military. A second limitation at IR wavelengths is that a substantial part of the IR region is occluded by a large absorbance band due to excitation of vibrational states of the water molecules present in the air.

8. Explain or Teach These Concepts to a Friend

1. Draw a three dimensional color space of the type that Maxwell devised: one axis represents the degree of activation of the S cones, a second axis represents the degree of activation of the M cones, and the third axis represents the degree of activation of the L cones. What set of points in this three-dimensional space corresponds to each of the pure wavelengths along the spectrum? What set of points corresponds to white light? What set of points corresponds to the family of colors between saturated red and white (i.e. all of the shades of pink)
2. Explain how amino acid sequence similarities and differences can be used to construct “tree” diagrams of evolutionary relatedness (i.e. a diagram where the lengths of the branches represent the extent of sequence divergence, and the point where two branches join together represents the most recent common ancestral sequence). Historical note: Charles Darwin drew the first “tree” diagram of species

diversity, and Linus Pauling conceived of the idea that this approach could be applied to protein sequences.

9. Research the Literature on Your Own

1. There is at least one visual task that dichromats perform better than trichromats. Read the paper listed below and discuss this experiment and its evolutionary implications:
2. Morgan MJ, Adam, A, Mollon JD (1992) Dichromats detect colour-camouflaged objects that are not detected by trichromats. Proceedings of the Royal Society (London) Series B (Biological Science) 248: 291-295.
3. On the web site of the National Center for Biotechnology Information at the National Institutes of Health (NIH), one can find the amino acid sequence files for rhodopsin and the cone pigments from various species, including humans. In the collection of protein sequences (click on “Protein”) the accession numbers for these sequences are: human rhodopsin (NP_000530), human S pigment (NP_001699), human M pigment (NP_000504), human L pigment (NP_064445). Use the “BLAST” program on this web site to search the database with each of the human rod and cone pigment sequences to see how closely they resemble each other and the sequences of visual pigments from other species.
4. There are many examples of related proteins encoded by gene families that have expanded by duplication and sequence divergence. Examples include the adult, fetal, and embryonic hemoglobins, and the more distantly related myoglobin and neuroglobin proteins. In what ways do the divergent globin sequences contribute to their specialized functions?