Jeff's View

My two blues

My favorite color is the blue I see with my left eye. I am also the happy owner of a right eye, but matters turned messy when surgeons replaced my cloudy eye lenses with sparkling pieces of polyacrylamide. That worked like a charm, but my right eye acted up and needed still other high-tech interventions. I came out of all this in one piece, but with two blues. On a cloudless day, my left eye shows me a violet-tinged blue sky whereas my right eye shows me one with a touch of gray. I wish I could explain it to you better, but how can I describe color? I might as well try to describe to you my wife's favorite perfume.

Color schizophrenia is not without its perks. It always reminds me that color, unlike shape or texture, is not an inherent feature of objects, but my way of sensing how they reflect or filter electromagnetic radiation. Another bonus is my belief that only I can appreciate our granddaughter's striking blue eyes – at least until, years from now, some young man will challenge me on that.

I can see only a tiny sliver of the immense spectrum of electromagnetic radiation that bathes the universe. This spectrum spans some 16 orders of magnitude – from the 10 km long radio waves used by our military all the way to 10^{-12} m long γ-rays emitted by disintegrating atoms or exploding stars. Life deals mostly with the wavelengths between about 300 and 1000 nm. This range includes the ultraviolet (below 400 nm), which I, unlike some insects, cannot see; the spectrum from blue to green to red (400 to about 750 nm), which I and many other organisms perceive as light; and the infrared (above 800 nm), which some animals can see, but I feel only as heat.

Blue may have been the first color life saw. Before cells came up with photosynthesis, sunlight with its harmful ultraviolet rays was a threat to them. To avoid it, early cells (the *archaea*) developed a blue-light sensor which controlled the cells' propellers so that the cells could swim away from blue light. This sensor has two parts. One is the colorless protein *archaeo-opsin*, whose polypeptide chain of about 250 amino acids is firmly stitched into the cell membrane, spanning it seven times. The other part is a yellowish small molecule with an absorption maximum of 374 nm and six conjugated double bonds. To chemists it is all-*trans*-retinal, but it is basically a vitamin A aldehyde. It is firmly attached to a lysine residue in one of the protein's membrane-spanning regions. When retinal binds to *archaeo-opsin*, it changes its color so that the resulting binary complex – the *archaeo-rhodopsin* – absorbs light best at around 480 nm. This absorption peak is at longer wavelengths than that of free retinal, but still in the blue region of the spectrum. When blue light hits *archaeo-rhodopsin*, it flips one of the double bonds of retinal from the straight *trans* to the bent *cis* conformation, pushes a proton within the protein from one place to another, and changes the shape of the entire *archaeo-rhodopsin* molecule. In a domino-like effect, this shape change ripples through neighboring proteins, which eventually transmit the light signal to the cell's propellers.

The light-driven proton movement within this blue light-sensing *archaeo-rhodopsin* may have inspired cells to convert the sensor (or an evolutionary ancestor) into an energy-capturing solar cell. By fiddling with the amino acid sequence, they shifted the absorption peak towards orange, closer to the sun's maximal energy output on the earth's surface. They also made the light push protons out of the *archaeo-rhodopsin* and all the way across the cell membrane. Because the cell membrane is an electric insulator, the positively charged protons were trapped outside the cell, capturing light energy as a transmembrane proton gradient. Cells probably already had a membrane enzyme that used the energy of ATP hydrolysis to pump protons out of the cell. By working in reverse, this enzyme could make ATP by letting the protons that had been pumped out by the spiced-up *archaeo-rhodopsin* flow back into the cell. Thanks to this sleek new machine, cells could now tap the energy of sunlight.

Perhaps I am telling the story backwards. Perhaps the proton-pumping *archaeo-rhodopsin* came first, and the blue light-sensing variety came later. Sequence comparisons do not reveal a clear-cut genealogy, but in either scenario, cells faced a tricky problem: they wanted orange light to power their proton pump, but did not want too much noxious blue light. To solve this problem, they modified the blue light sensor so that it absorbed best in the orange region of the spectrum and could steer them towards orange light. However, once the protein had seen orange light, it turned into a blue light sensor, which could warn cells to dive for cover when there was too much blue light. Life had invented color vision.

Proton-pumping *archaeo-rhodopsin* is one of the most ingenious devices life ever invented. Why did life not develop it further, and put it into all the modern light-capturing organisms of today? Perhaps the machine was too simple. It could not furnish the reducing power cells needed to synthesize their building blocks, and its light capture was not all that efficient. Life is always on the prowl for better things, and when it stumbled upon chlorophyll, it held on to it. Chlorophyll absorbed sunlight even better than retinal and also allowed the evolution of systems that extracted reducing electrons from water. Retinal pioneered photosynthesis, but chlorophyll walked away with it.

Modern eukaryotes draw their energy mostly from respiration or from chlorophyll-based photosynthesis, or from both. They no longer have much use for proton-pumping *archaeo-rhodopsin*. The protein persists in today's archaea and in many marine bacteria where it backs up the more modern chlorophyll-based photosynthesis. Good old *archaeo-rhodopsin* may still scoop up as much as one fifth of the photons that feed our oceans' bacteria.

Light-sensing *archaeo-rhodopsin*, however, was headed for bigger things. In its relentless quest for vision, life tested the protein successfully as a light sensor in some eukaryotic algae and molds. These cells respond to light, whereas mutants lacking the protein do not – they are blind. As eukaryotes became more sophisticated, they changed the retinal slightly to 11-*cis*-all-*trans* retinal. They also retooled the protein (or a molecular ancestor) by changing its amino acid sequence and

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tacking on a bulky loop that sticks out from the membrane into the cytosol. Thanks to this loop, the new *rhodopsin* could interact with the cytosolic components of the eukaryotic signal transducing systems. These changes altered the protein almost beyond recognition; only the tell-tale seven transmembrane spans still reveal its archaeal roots.

Once life had seen color, it was hooked. The large eukaryotic genomes were exciting new playgrounds for experimenting with ever better color perception and eukaryotes exploited them to the fullest. Like the archaea before them, they developed a two-color vision system by attaching modern 11-*cis*-retinal to two slightly different variants of eukaryotic opsin. Already more than 800 million years ago, this eukaryotic *rhodopsin* system allowed animals to distinguish blue (below 500 nm) and yellow (above 500 nm) – the animals were dichromats. Later on, insects and higher animals duplicated the gene for the yellow sensor and then mutated one of the two copies, so that the mutated copy responded to red or green. Further modifications of *rhodopsin* led to systems that could see still more colors: bees, many fish, reptiles and birds can distinguish four colors, and many butterflies as many as five – from deep red all the way into the ultraviolet. Some animals can even peer into the infrared. In order to see in dim light, most animals also acquired yet another type of *rhodopsin* that is extremely light-sensitive, but can not distinguish between different colors. About 400 million years ago, many animals had three to five different color sensors as well as a rhodopsin for dim light. Early mammals that hunted mostly at night did not need to see many colors and became dichromats again. It was only 35 million years ago that ancestors of primates and humans re-invented mammalian three-color vision, perhaps because it helped them distinguish ripe from unripe fruits against a background of confusing foliage. Dichromats would have a lot of trouble to do this. There must be many other amazing things we still do not know about how animals see color. In fact, the only systems we really understand in detail are our own and those of primates.

My retinas have three types of cone-shaped photoreceptor cells that have broad and overlapping absorption peaks in the blue, green and red regions of the visible spectrum. That makes me a trichromat. The combination of the relative signal strengths from these three photosensors lets me see more than two million colors. Humans and primates are the only mammals that can see so many colors. Most other vertebrates with their two color sensors can distinguish only about 10 000 colors – that's six-fold less than on today's mobile phone displays. My color receptors are very good at resolving colors and fine detail, but need lots of light. When it gets dim, they fall silent and leave the field to my rod-shaped photoreceptors that are very light-sensitive, but wake up very slowly and give me only low resolution and no more than 200 shades of gray. Have you ever wondered why many bars and restaurants have such crummy lighting? Customers love it because it hides their facial wrinkles and gray hair. Within my retina, rods and cones form an uneven mosaic, and the cones already compare the color signals among each other before they send their joint report to my brain for final analysis. Both tissues work very hard at it: my retina consumes more energy per gram than any other of my tissues, and my brain is not far behind. That's why both of them tend to go bad with age. Because they are so energy-hungry, they ferment glucose to lactic acid even when there is plenty of oxygen around. No other part of my body does that. At least that's what I hope, because the only exceptions to this rule are cancer cells.

Overall, I have about ten to twenty times more rods than cones. My retina's center is exceptionally rich in shape-discriminating cones and I rely on it for my most acute vision. One of my colleagues has once called the retina's center the most valuable square millimeter of the human body. This was before young ladies started to stud their navels with diamonds, but he is still right.

My gene for blue-sensing opsin sits far apart from my other opsin genes on chromosome 7. But my genes for the red-sensing and green-sensing opsin originally arose by duplication of an ancestral gene for a yellow sensor and therefore still sit next to each other on my single X chromosome. As all our opsin genes have similar sequences, this spells trouble for women with their two X chromosomes. When they produce egg cells, they occasionally replace the single gene for red-sensing opsin by two genes for the green-sensing opsin on the same X chromosome, or vice versa. The result is an X chromosome that has only two green sensors, or two red sensors. That's no problem for the daughters, because they still get the missing color sensor from their father's X chromosome, which is likely to be normal. But the sons with their single X chromosome have a 50% chance of being left with only two green sensors, or only two red sensors. Even though the two greens or the two reds will not be identical, they are usually so similar that these unlucky fellows have only two color sensors: for blue and red, or for blue and green. We call them color-blind, but they are really dichromats who see fewer colors than the rest of us. 'Color-blindness' was clearly described only in 1777 by Joseph Huddart in his classic *An account of persons who could not distinguish colours*. Two-color vision afflicts 8% of Caucasian men, but hardly any women. Keep that in mind when you prepare your next plenary lecture, because about 80 males in an audience of 2000 will see your colored power points quite differently than you imagine. Yet dichromacy also has its upsides. The US army has found 'color-blind' recruits useful as sharp-shooters or scouts because they are not as easily fooled by multi-colored camouflage. For you this means that those 80 male listeners won't be fooled by your multi-colored power points and will take shots at any weak data.

The mix-up among the neighboring genes for red-sensing and green-sensing opsin during egg cell formation can sometimes create an X chromosome that encodes two green sensors whose absorption peaks are as much as 10 nm apart. A daughter inheriting this X chromosome should have four distinct color sensors: the blue sensor encoded on chromosome 7, two different green sensors from the mother's messed-up X chromosome, and a normal red sensor from the father's normal X chromosome. But could she plug that extra green sensor into her neural network and actually see more colors than normal mortals? Would she be a *functional* tetrachromat?

It seems so. When the gene for the human red-sensing opsin is expressed in mice (which are dichromats and normally lack our red sensor), the human sensor populates the animal's retina in a random mosaic fashion and responds electrophysiologically to its appropriate color. This tells us that at least the retina knows what to do with the extra sensor. But what about the human brain?

About ten years ago, British scientists set out to hunt for 'Ms Tetrachromat' among 14 mothers whose sons were color-

blind because they had inherited duplicate green or red sensors. The women were asked to mix red and green lights with a joystick-controlled device in order to recreate a particular hue of yellow-orange that was outside the working range of the human blue sensor. As expected, normal trichromats, having only their red and green sensors to go on, found many matching combinations. But one particular women (the 57 year old 'Ms. M') was exceptionally fussy about color matching; she found only a single match that satisfied her. According to genetics, she had an extra green sensor peaking between green and red, and apparently she used it well. Still, the existence of tetrachromatic women is not yet solidly established, because it is so hard to do conclusive experiments. Scientists may not be normal people, but most of them are still trichromats and have no objective way of telling whether a test subject's choice of color match is correct.

What would life be like for tetrachromatic women? At times quite a pain, because most photos, movies or TV screens would show them the wrong colors. But they might well be phenomenal at playing computer games, surfing the internet, or analyzing colored diagrams because they might feed their brain color-coded information through four, rather than three channels. We dumb males could only watch them in awe. But before we decide to marry one of them we should remember that her sons would have a 50% chance of being color-blind.

Many people have slightly abnormal color sensors that may affect their color perception. The differences are usually minor, but there is no doubt that many of us see colors in a unique way which we cannot share with others. When it comes to seeing color, each of us is very much alone. The known combinations of opsin with retinal can only produce color sensors covering the spectral range from 345 to 610 nm. But rare mutations that change opsin, retinal, or some of their partner molecules could well extend this range. And the immensely complex circuitry of our signal transduction systems may allow mutations that distort the balance of color signals still further. Such rare human mutants might have exceptional night vision, or be 'mind readers' because they perceive minute fluctuations of other peoples' skin color.

Why do I see two different blues? Even when my eyes were still faultless, they had fewer 'blue' cones than 'red' or 'green' cones – like all human eyes. The ratio of blue cones to red and green cones was particularly low in my retina's center because nature tried to compensate for the chromatic aberration of the natural lenses I once had. I suspect that the operations on my right eye destroyed too many of the rare blue cones in the retina's periphery. Surgeons would have to open up my eyes to make sure – but I won't let them do this. I am perfectly happy with the way things are – and that includes my two blues.

Ever since Isaac Newton's published his 1671 classic *New Theory of Colors*, our perception of color has intrigued many of our greatest scientific minds. When light proved to be just one form of electromagnetic radiation, it still remained puzzling why we cannot create some colors by mixing the others. These 'primary colors' seemed to imply that the electromagnetic spectrum obeyed some hierarchy which, once understood, would give us fundamental insights into the nature of light. But the three primary colors are just the shadows of our three color sensors projecting on the monotonous immensity of the electromagnetic spectrum. They are products of our imagination. In the end, understanding primary colors and color vision in general has told us much less about the nature of light than about the nature of ourselves.

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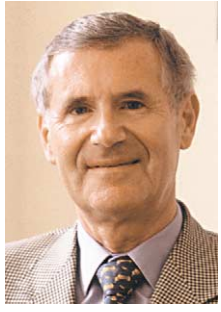

Gottfried Schatz
Biozentrum, University of Basel,
Basel, Switzerland
E-mail address: gottfried.schatz@unibas.ch