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Photopigment genes, cones, and color update: disrupting the splicing code causes a diverse array of vision disorders

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Abstract

The human long- and middle-wavelength sensitive cone opsin genes exhibit an extraordinary degree of haplotype diversity that results from recombination mechanisms that have intermixed the genes. As a first step in expression, genes—including the protein coding exons and intervening introns—are transcribed. Next, transcripts are spliced to remove the introns and join the exons to generate a mature message that codes for the protein. Important information necessary for splicing is contained within exons, and is overlaid by the protein code. Intermixing the long- and middle-wavelength sensitive cone opsin genes has disrupted the splicing code, leading to exclusion of some exons from the mature message and is associated with several vision disorders including nearsightedness, cone dystrophy, and color vision deficiencies.

Graphical Abstract

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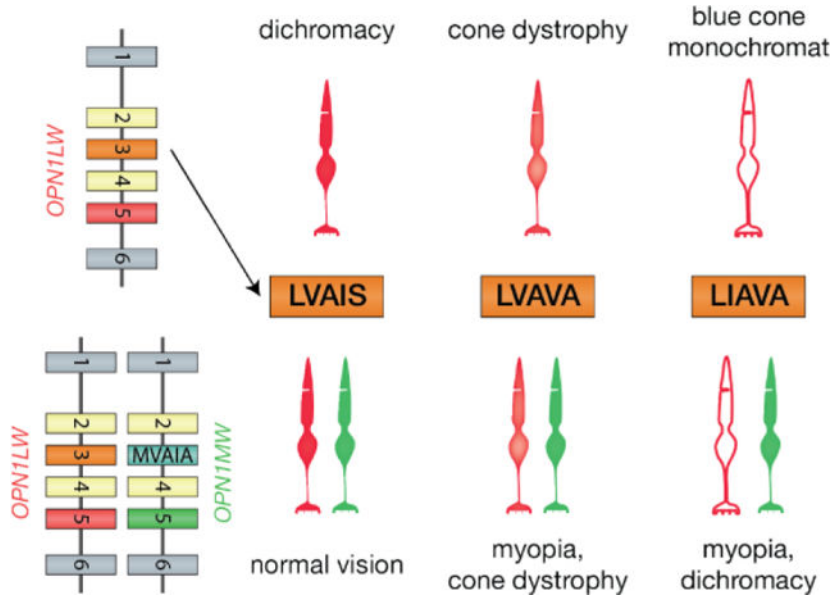
Maureen Neitz and Jay Neitz have significant financial interest in the company Waveshift and its subsidiaries, SightGlass and Visu, and receive royalties from the University of Washington for intellectual property, including patents, licensed to these companies. Sara Patterson: none



Introduction

Humans with normal color vision have three types of cone photoreceptor that are classed according to their relative spectral sensitivities as short- (S), middle- (M) and long- (L) wavelength sensitive. Neural comparisons between their quantal catches give rise to six main hue percepts – red, green, blue, yellow, black and white – and combinations thereof yield more than a million distinguishable colors [1–3]. The color palette is greatly reduced for individuals with inherited red-green color vision deficiencies. Rearrangements and mutations of genes encoding the protein component (opsin) of cone photopigments are the primary cause of inherited color vision deficiencies (dichromacies and anomalous trichromacies) [4–6]. Except at very low light levels when rods are active—all vision, not just color vision—is based on cones [2]. Here we briefly review the role of genetic variation in the cone opsin genes in inherited color vision deficiencies, including recent discoveries of mutations that cause defective splicing and result in several vision disorders including cone dysfunction, nearsightedness (myopia), cone dystrophy, blue cone monochromacy, and dichromacy.

Genetics of Common Inherited Color Vision Deficiencies

Inherited color vision deficiencies are categorized according to the number of functional cone types contributing to vision (Figure 1). All are caused by mutations that alter the complement of functional cone opsins expressed. The L, M and S-cone opsin genes, designated *OPN1LW*, *OPN1MW*, and *OPN1SW*, respectively, have similar organizations. Inherited color vision deficiencies associated with mutations in *OPN1SW* are rare and will not be discussed here. *OPN1LW* and *OPN1MW* are on the X-chromosome. The number of X-chromosomes per cell is two for females and one for males, but female cells—including L- and M-cones—express genes from one of the two X-chromosomes.

Trichromatic color vision appeared recently in mammalian evolution, and is found only in primates. In Old World Primates, including humans, *OPN1LW* and *OPN1MW* gene expression is segregated to separate cone populations, allowing males and females alike to have trichromatic color vision. In non-human Old World Primates, L- and M-cone photopigments are stereotyped within a species, differing at ~18 amino acid positions, seven of which shift the spectra (Figure 2). Presumably differences not involved in spectral tuning were necessary to preserve correct splicing or to stabilize the opsins after they diverged.

Unlike in non-human Old World Primates, human *OPN1LW* and *OPN1MW* genes vary in copy number and exhibit genetic variability because recombination mechanisms intermix the genes and redistribute them to form new gene arrays that underlie inherited red-green color vision deficiencies. Relaxation of selection against inherited red-green color vision deficiencies presumably allowed opsin gene arrays underlying these defects to propagate, as evidenced by the 15% prevalence of female carriers of inherited red-green color vision defects in present day populations [6].

Figure 3 illustrates recombination events responsible for generating opsin gene arrays that cause inherited red-green color vision defects. In females, exchange of genetic material mediated by a cross-over between an *OPN1LW* gene and an *OPN1MW* gene on different X-chromosomes produces two new arrays that both contain intermixed, chimeric genes. The example in Figure 3A shows recombination between two arrays that each have one *OPN1LW* and one *OPN1MW* gene. The genes from the parental chromosomes are redistributed, creating a new array with three genes, and another with one gene. Both arrays have chimeric genes. The chimeric gene in the three-gene array derives exon 5 from the parental *OPN1LW* gene, and it encodes an L-class pigment. Only the leftmost two genes in the array are expressed, so the array underlies deutan-type color vision. Whether the array underlies dichromacy or anomalous trichromacy depends on whether spectral tuning sites in exons 2, 3 and 4 differ between the two L pigments. The one-gene array carries a chimeric gene that encodes an M-class pigment because it derives exon 5 from a parental *OPN1LW* gene, and it will confer protanopia.

The region between the genes is nearly identical to the region following the last gene in the array. Recombination between these two regions (Figure 3B) does not intermix *OPN1LW* and *OPN1MW*, but does redistribute the genes such that one new array carries one gene, which encodes an L pigment and will confer dichromacy (deuteranopia). The other carries *OPN1LW* followed by two *OPN1MW* genes and will confer normal color vision.

Variation in the number of opsin genes on the X-chromosome in present day populations allows recombination between the middle gene in a three gene array and the first gene in a two gene array as illustrated in Figure 3C. This recombination gives rise to a new array in the which the first gene is chimeric followed by an OPN1MW gene. The chimeric gene derives exon 5 from a parental *OPN1MW*, and thus encodes an M class pigment. The array underlies a protan color vision deficiency, dichromacy or anomalous trichromacy, depending on whether spectral tuning sites encoded by exon 3 and 4 differ in the two genes. The other product of the recombination illustrated in Figure 3C is the same as that underlying deutan color vision shown in Figure 3A.

Because expression of opsin genes from the two X-chromosomes is segregated to separate cone populations, a female will have an inherited red-green color vision defect only if both of her X-chromosomes carry the same category of inherited red-green defect —protan or deutan. If one X-chromosome carries a protan defect and the other a deutan defect, she will have L- and M-cones and normal color vision.

Intermixing *OPN1LW* and *OPN1MW* Causes Inherited Color Vision Defects, Cone Dysfunction, and High-Myopia.

Variability in the nucleotide sequence (haplotype diversity) of exons 2 – 4 of *OPN1LW* and *OPN1MW* has been evaluated with regard to selective pressure for red-green color vision [7–10]. However, cones provide more than color vision. Importantly, they mediate high acuity spatial vision and achromatic black-white vision [11], as was recently illustrated when color percepts elicited by adaptive optics-mediated micro-stimulation of individual cones, revealed that the majority of L- and M-cones elicit the percept of white [2]. A small subset of L- and M-cones in the vicinity of S-cones elicited red, green or blue color percepts [12]. Thus, most L- and M-cones mediate high-acuity achromatic spatial vision. After birth, human eyes undergo emmetropization, a controlled axial elongation, governed by a feedback mechanism in which L-and M-cones play a critical role so that the eye stops growing when the length is optimally matched to the power of the optical components for high acuity vision. Nearsightedness (myopia) results if the eye grows too long.

An exon 3 haplotype was recently identified as the cause of Bornholm Eye Disease a syndrome characterized by high-myopia, and inherited red-green color vision defects [13,14]. The haplotype is abbreviated LVAVA for the amino acids at the polymorphic positions encoded by exon 3 which are Leucine 153, Valine 171, Alanine 174, Valine 178, and Alanine 180. Bornholm Eye Disease was genetically linked to the first officially designated high-myopia gene, *MYP1*, located at Xq28, the same location as *OPN1LW* and *OPN1MW* [15]. The LVAVA haplotype has been demonstrated independently by several labs as a cause of high-myopia [13,16–18].

Initially it was assumed that if *OPN1LW*/*OPN1MW* were responsible for Bornholm Eye Disease, all affected individuals would have the same color vision phenotype. However, they do not because the role of the cone opsin genes in myopia is independent of their role in color vision. That is, the color vision phenotype in Bornholm Eye Disease individuals with a deutan defect is accounted for by their opsin gene arrays in which the first two genes encode L-class pigments such as the deutan array in Figure 3A. They have high-myopia because one of the first two genes has the LVAVA haplotype, which causes a splicing defect and produces cones with dramatically reduced amounts of photopigment [14]. These patients have two submosaics of L-cones, one expressing the LVAVA opsin and another expressing a normal *OPN1LW* haplotype and this appears to interfere with emmetropization. We hypothesized that the associated high-myopia is the result of an erroneous contrast signal initiated at the level of the cones [14] due to dramatically different amounts of photopigment in the two subpopulations of cones. Similarly, the protan color viison in the second Bornholm Eye Disease family is accounted for by their opsin gene array having two *OPN1MW* genes such

as the protanomalous array in Figure 3C, and they have high-myopia because one of the *OPN1MW* genes specifies the LVAVA haplotype, the other specifies a normal variant. Cones expressing a gene encoding the LVAVA haplotype are associated with cone dystrophy [19,20].

The first *OPN1LW*/*OPN1MW* exon 3 haplotype associated with cone dysfunction was abbreviated “LIAVA” and differs from LVAVA in encoding isoleucine instead of valine at positon 171. This haplotype causes a splicing defect and appears to give rise to “empty cones” which are stable over many years [19–21]. When a normal haplotype is expressed in a separate population of cones, the LIAVA haplotype is associated with high-myopia particularly when the larger fraction of L-/M-cones are those expressing the LIAVA haplotype [17], and with dichromacy. LIAVA is also associated with blue cone monochromacy when all but the S-cones express an LIAVA opsin gene haplotype.

Intermixing the *OPN1LW* and *OPN1MW* Genes Disrupts the Splicing Code

The LIAVA- and LVAVA-exon 3 haplotypes fail to be recognized during pre-messenger RNA (pre-mRNA) splicing [22,23]. Genes are initially transcribed in their entirety to generate pre-mRNA. Splicing removes the introns from the pre-mRNA and joins the exons to form the mature mRNA, which carries a contiguous protein coding sequence. When the splicing apparatus fails to recognize an exon, it is excluded from the mature mRNA [14,22,23], which disrupts the protein coding sequence. Opsin mRNA lacking exon 3 likely is targeted for destruction and not translated into protein.

The 3’ and 5’ consensus splice sites at the intron/exon junctions in pre-mRNA contain insufficient information for correct splicing [24]. Additional information is provided by 6–8 nucleotide sequences that serve as enhancers of splicing (Figure 4). When located in exons, these serve a dual role, providing information needed for splicing and for making a functional protein. Intermixing the *OPN1LW* and *OPN1MW* exon 3 sequences has created haplotypes that disrupt the splicing code. For example, the LVAIS haplotype is normal-splicing haplotype, not associated with vision disorders. It contains sufficient information for exon 3 to be included in the mRNA always [23]. Cones expressing this haplotype contain the maximum amount of photopigment. Splicing enhancers in LVAIS associated with I178 and S180 are disrupted in LVAVA, and nucleotide changes associated with V178 and A180 in LVAVA introduce splicing silencers [25]. Splicing silencers are also short nucleotide sequences and, when in exons, they promote exon skipping. The splicing apparatus does not always recognize the LVAVA-exon 3, and produces a mixture of exon 3-skipped and full-length mRNA (Figure 4). Functional photopigment is made only from the full length mRNA so cones expressing the LVAVA haplotype have a reduced amount of photopigment. Splicing enhancers associated with V171, I178 and S180 in LVAIS are all disrupted by nucleotides associated with I171, V178 and A180 in LIAVA, and silencers are created at all three locations in LIAVA [25]. LIAVA-exon 3 is excluded from the mRNA most, if not all of the time, and thus, the LIAVA haplotype gives rise to empty cones. It is not known whether exon 3-skipping is caused by disruption of enhancers or activation of silencers, or some combination of the two.

Potential for Splicing Differences to Alter Photopigment Optical Density in Cones.

In anomalous trichromacy (Figure 1), color vision is usually mediated by a difference in peak sensitivity of the underlying L or M-cones; but can be mediated by two cone populations that have the same peak sensitivity but different optical densities [26]. The discovery of exon 3-skipping haplotypes of *OPN1LW* and *OPN1MW* provides a potential mechanism for generating optical density differences that could underlie anomalous trichromacy.

Conclusions

For most of the 30 years since the molecular genetics of inherited color vision deficiencies was elucidated, ideas about evolutionary advantages or disadvantages of cone opsin gene variation, including comparisons between humans and non-human Old World Primates, have considered only the potential effects on color vision and protein structure and function.

The human *OPN1LW* and *OPN1MW* genes have been intermixing over the course of human evolution, presumably due to reduced selection against disorders associated with recombinant arrays. Compared to other Old World Primates, human innovations make us less dependent on normal color vision and non-myopic vision for survival. The recent discovery that intermixing the opsin gene sequences produces haplotypes that cause splicing defects which lead to a much broader spectrum of vision disorders, not just inherited red-green color vision defects, is a revelation that is expected to ultimately have a significant impact on our understanding of cone-based vision disorders and on the development of treatments for them.

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** of outstanding interest

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Highlights Normal color vision requires short-, medium- and long-wavelength sensitive cones

- Inherited color vision deficiency reduces the number of cone types
- Cones are responsible for color vision and high acuity spatial vision
- L- and M-cones play a critical role eye growth
- Intermixing *OPN1LW* and *OPN1MW* genes causes splicing errors and vision defects

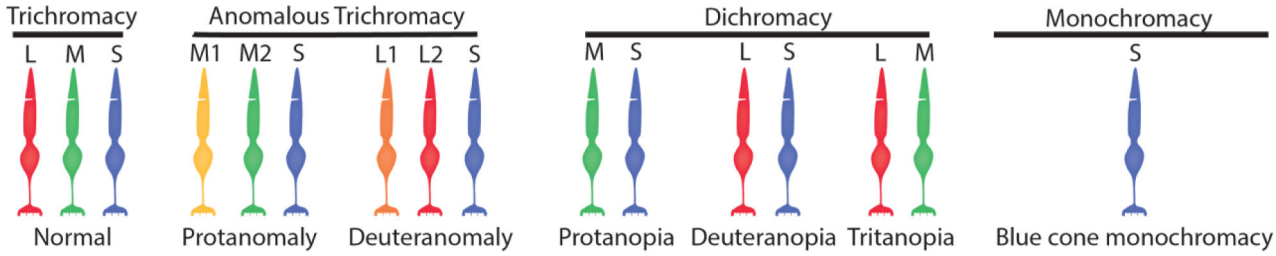

Figure 1.

Complement of cones types in normal color vision and inherited color vision deficiency. Protanopia and protanomaly lack contribution from L-cones, deuteranopia and deuteranomaly lack contributions for M-cones, tritanopia lacks contributions from S-cones, and blue cone monochromacy lacks contribution from L- and M-cones.

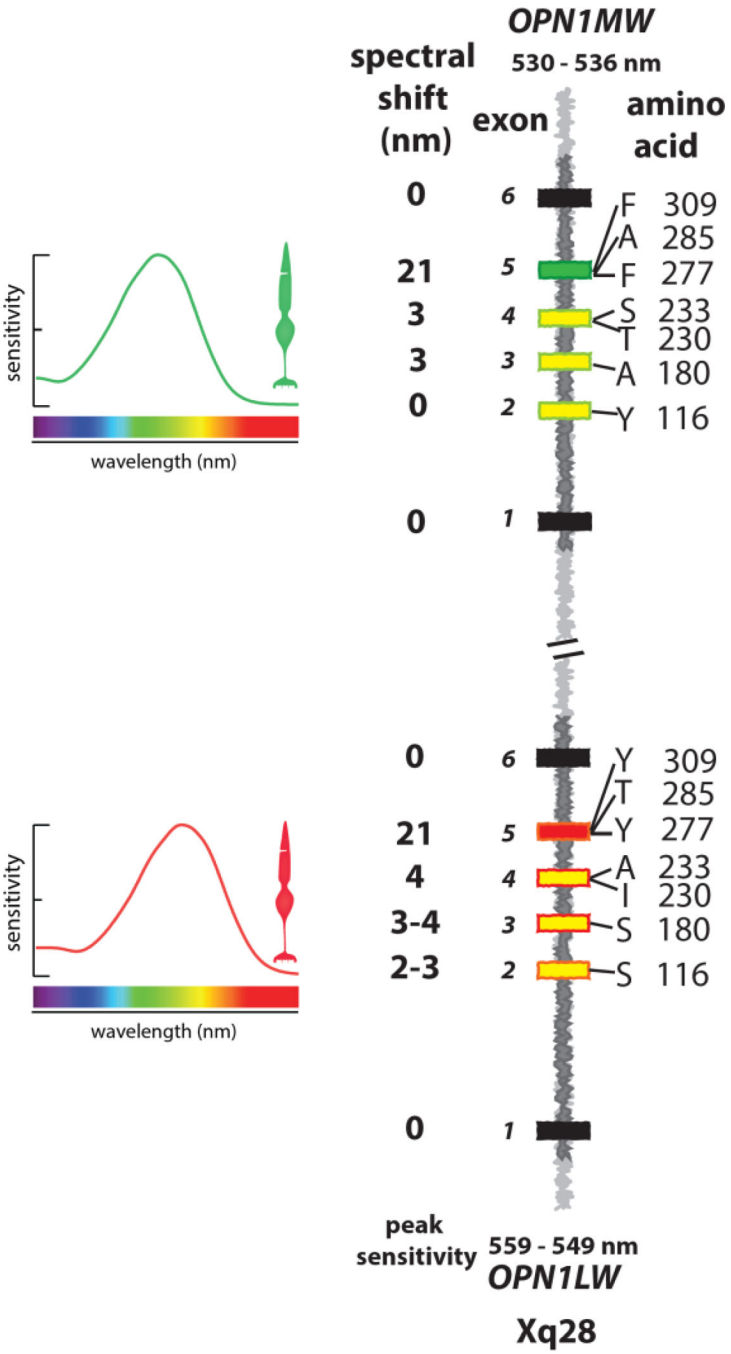

Figure 2.

Organization of *OPN1LW* and *OPN1MW* and spectral tuning of L- and M-cones. *OPN1LW* and *OPN1MW* lie in tandem on the X-chromosome at Xq28 and are nearly identical in sequence. Exons are black, yellow, green and red boxes. Exons 1 and 6 are invariant (black boxes), exon 5 (red and green boxes) encodes amino acids that tune the spectra and define whether the encoded pigment is of the L-class or M-class. Exons 2, 3 and 4 specify amino acids that tune the spectra but do not define whether the encoded pigment is of the L- versus M-class. These are indicated using the single letter amino acid code followed by the codon/

amino acid position. In this illustration, the amino acids encoded by exons 2, 3 and 4 that shift the spectrum toward the long-wavelength (magnitudes given in figure) are shown in *OPN1LW* (yellow boxes with red outlines) and those that shift the spectrum to the short-wavelengths are shown in *OPN1MW* (yellow boxes with green outlines). Depending on the combination of amino acids encoded by exons 2–4 L-cone peak sensitivity ranges from 549 to 559 nanometers (nm). Depending on exon 3 and 4, M-cone peak sensitivity ranges from 530 to 536 nm. The single letter amino acid code is A=alanine, F=phenylalanine, G=glycine, I=isoleucine, L=leucine, P=proline, Q=glutamine, R=arginine, S=serine, T=threonine, Y=tyrosine.

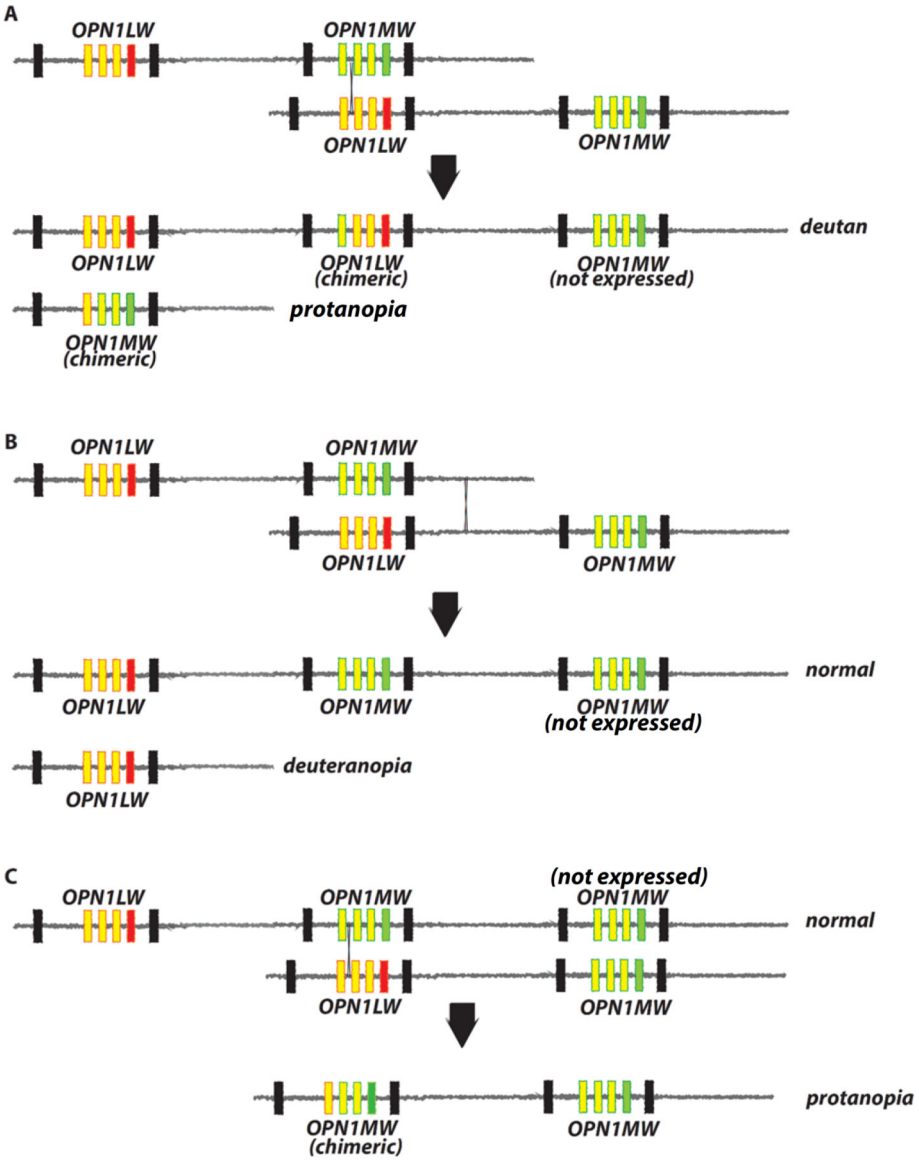

Figure 3.

Recombination intermixes the *OPN1LW* and *OPN1MW* gene sequences, generates variability in the number of opsin genes on the X-chromosome, and causes red-green color vision defects. (A)-(C) The top two parental X-chromosome opsin gene arrays exchange genetic material at the location indicated by the thin black X. All parental arrays confer normal color vision. Parental *OPN1LW* genes have a red box for exon 5 and yellow boxes with red outlines for exons 2–4. Parental *OPN1MW* genes have a green box for exon 5 and yellow boxes with green outlines for exons 2–4. Below the black arrows in (A)-(C) are the recombination products and the type of color vision they confer. The parental gene of origin for exons 2–4 in chimeric genes are indicated by the color of the outline (red for *OPN1LW*, green for *OPN1MW* origin), and by the color of the box for exon 5 (red for *OPN1LW*, green for *OPN1MW)*.

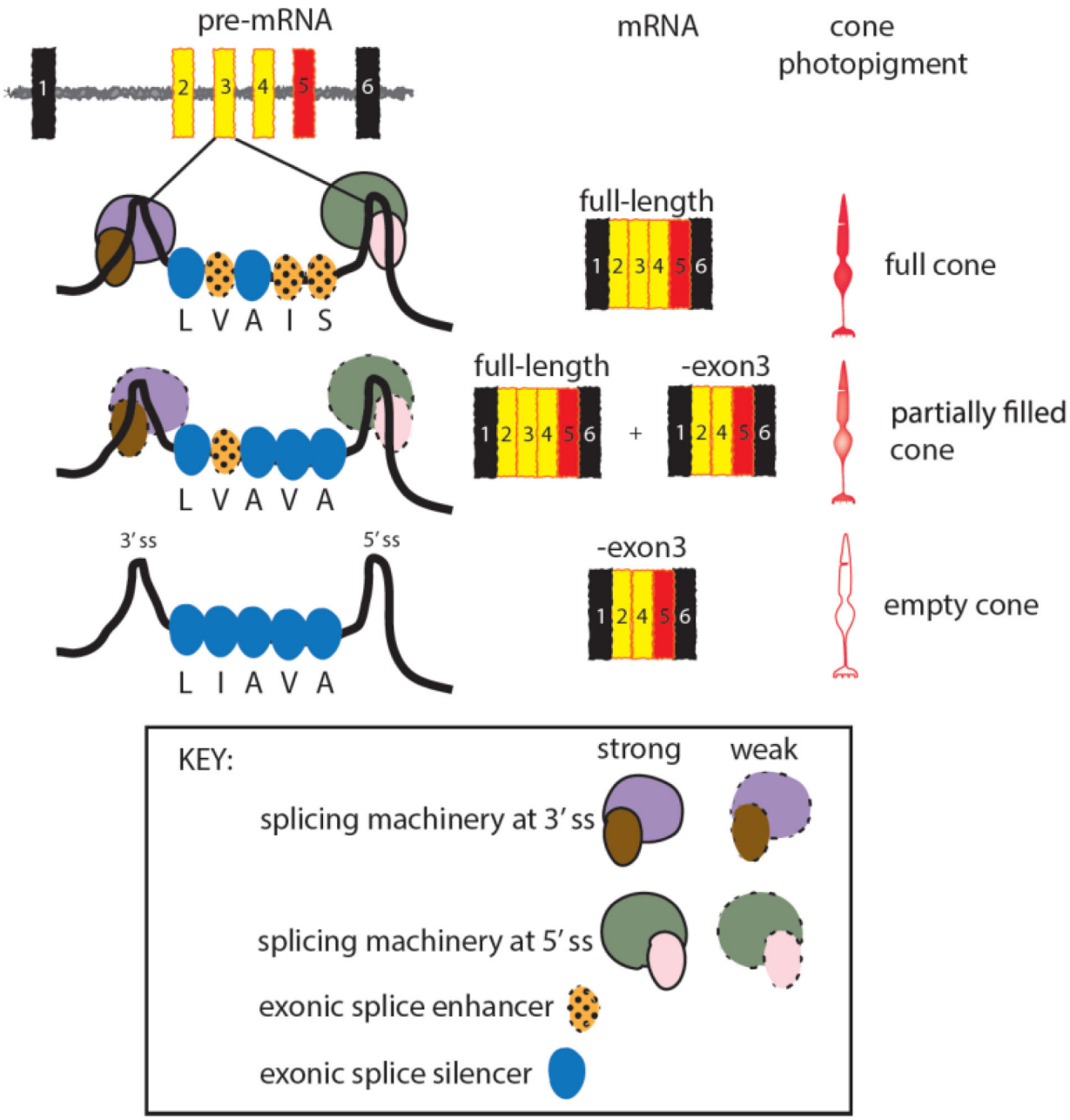

Figure 4.

Model for how nucleotide sequence variability in exon 3 alters splicing and the amount of photopigment in cones. The most common human *OPN1LW* exon 3 haplotype is abbreviated “LVAIS” for the amino acids specified at the five exon 3-encoded polymorphic amino acid positions (see Figure 2 single letter amino acid code). Nucleotide triplets specify the amino acid code, hexamers and octamers specify the splicing code and, within exons, can act as enhancers to promote exon inclusion in mRNA or act as silencers to inhibit exon inclusion. Components of the splicing machinery bind to enhancers and interact with the RNA-Protein complexes bound to the 5’ and 3’ splice sites at the intron/exon junctions and assist in including the exon in the mRNA. Exonic splicing silencers can block the binding of

proteins to enhancers or inhibit assembly of the splicing machinery, promoting exon exclusion. The LVAIS haplotype includes exon 3 most, perhaps all, of the time. LIAVA does not include exon 3 a detectable amount. LVAVA includes exon 3 some of the time. Based on observations in humans the amount of photopigment in the cone correlates to the amount of full length mRNA [14].