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A mouse M-opsin monochromat: Retinal cone photoreceptors have increased M-opsin expression when S-opsin is knocked out

Lauren L. Daniele [a], Christine Insinna [a], Rebecca Chance [b], Jinhua Wang [c], Sergei S. Nikonov [d], Edward N. Pugh Jr. [a,*]

[a] Center for Neuroscience, University of California, Davis, United States
[b] Graduate Program in Neuroscience, University of Pennsylvania, United States
[c] Department of Dermatology, University of Pennsylvania, United States
[d] Department of Neuroscience, University of Pennsylvania, United States

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ABSTRACT

Mouse cone photoreceptors, like those of most mammals including humans, express cone opsins derived from two ancient families: S-opsin (gene *Opn1sw*) and M-opsin (gene *Opn1mw*). Most C57Bl/6 mouse cones co-express both opsins, but in dorso-ventral counter-gradients, with M-opsin dominant in the dorsal retina and S-opsin in the ventral retina, and S-opsin 4-fold greater overall. We created a mouse lacking S-opsin expression by the insertion of a Neomycin selection cassette between the third and fourth exons of the *Opn1sw* gene ($Opn1sw^{Neo/Neo}$). In strong contrast to published results characterizing mice lacking rhodopsin ($Rho^{-/-}$) in which retinal rods undergo cell death by 2.5 months, cones of the $Opn1sw^{Neo/Neo}$ mouse remain viable for at least 1.5 yrs, even though many ventral cones do not form outer segments, as revealed by high resolution immunohistochemistry and electron microscopy. Suction pipette recordings revealed that functional ventral cones of the $Opn1sw^{Neo/Neo}$ mouse not only phototransduce light with normal kinetics, but are more sensitive to mid-wavelength light than their WT counterparts. Quantitative Western blot analysis revealed the basis of the heightened sensitivity to be increased M-opsin expression. Because S- and M-opsin transcripts must compete for the same translational machinery in cones where they are co-expressed, elimination of S-opsin mRNA in ventral $Opn1sw^{Neo/Neo}$ cones likely increases M-opsin expression by relieving competition for translational machinery, revealing an important consequence of eliminating a dominant transcript. Overall, our results reveal a striking capacity for cone photoreceptors to function with much reduced opsin expression, and to remain viable in the absence of an outer segment.

1. Introduction

1.1. Photoreceptor viability and outer segment structure may depend on opsin expression

Vertebrate retinal photoreceptors initiate vision via a G-protein coupled receptor (GPCR) cascade whose molecular components are concentrated within their outer segments, whose renewal gives rise to a distinctive daily burden of transcription, translation, protein trafficking and turnover. The outer segments comprise a dense stack of lamellar membranes packed with opsin GPCR at a membrane density of ~25,000 μm^{-2}, among the highest of all membrane proteins, and are renewed at a rate of about 10% per day, (Hollyfield, 1979; Young, 1967, 1971). Rod photoreceptors require substantial opsin expression for the elaboration of functioning outer segments, and ultimately for cell viability: rods of mice from which rhodopsin has been genetically deleted ($Rho^{-/-}$) form only rudimentary cilia-like outer segments with no disc structure, and eventually undergo apoptosis, leading to severe retinal degeneration by age 2.5 months (Humphries et al., 1997; Lee, Burnside, & Flannery, 2006). Cone photoreceptors also fail to elaborate outer segments when their opsins fail to traffic normally, and subsequently undergo rapid degeneration (Zhang et al., 2008; Znoiko et al., 2005). These observations have led to the hypothesis that photoreceptor viability requires normal opsin expression and outer segment structure, but this has not been tested in cones.

1.2. Analysis of cone opsin knockouts is complicated by the coexpression of cone opsins in dorsal – ventral countergradients

In principle, this hypothesis could be tested in mice by knocking out one of the two distinct cone opsins, short-wave sensitive opsin (S-opsin; gene *Opn1sw*) or mid-wave opsin (M-opsin; *Opn1mw*).

* Corresponding author. Address: Department of Physiology, Center for Neuroscience, University of California, Davis, 1544 Newton Court, Davis, CA 95618, United States.
E-mail address: enpugh@ucdavis.edu (E.N. Pugh).

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However, the matter is complicated by the facts that most mouse cones co-express both opsins, and that the relative expression of the two opsins follows a dual dorso-ventral gradient, with M-opsin dominant in the most dorsal retina, and S-opsin dominant elsewhere (Applebury et al., 2000; Nikonov, Kholodenko, Lem, & Pugh, 2006; Szel, Lukats, Fekete, Szepessy, & Rohlich, 2000). Thus, understanding the consequences of deleting one of the cone opsins requires detailed consideration of the gradients of expression.

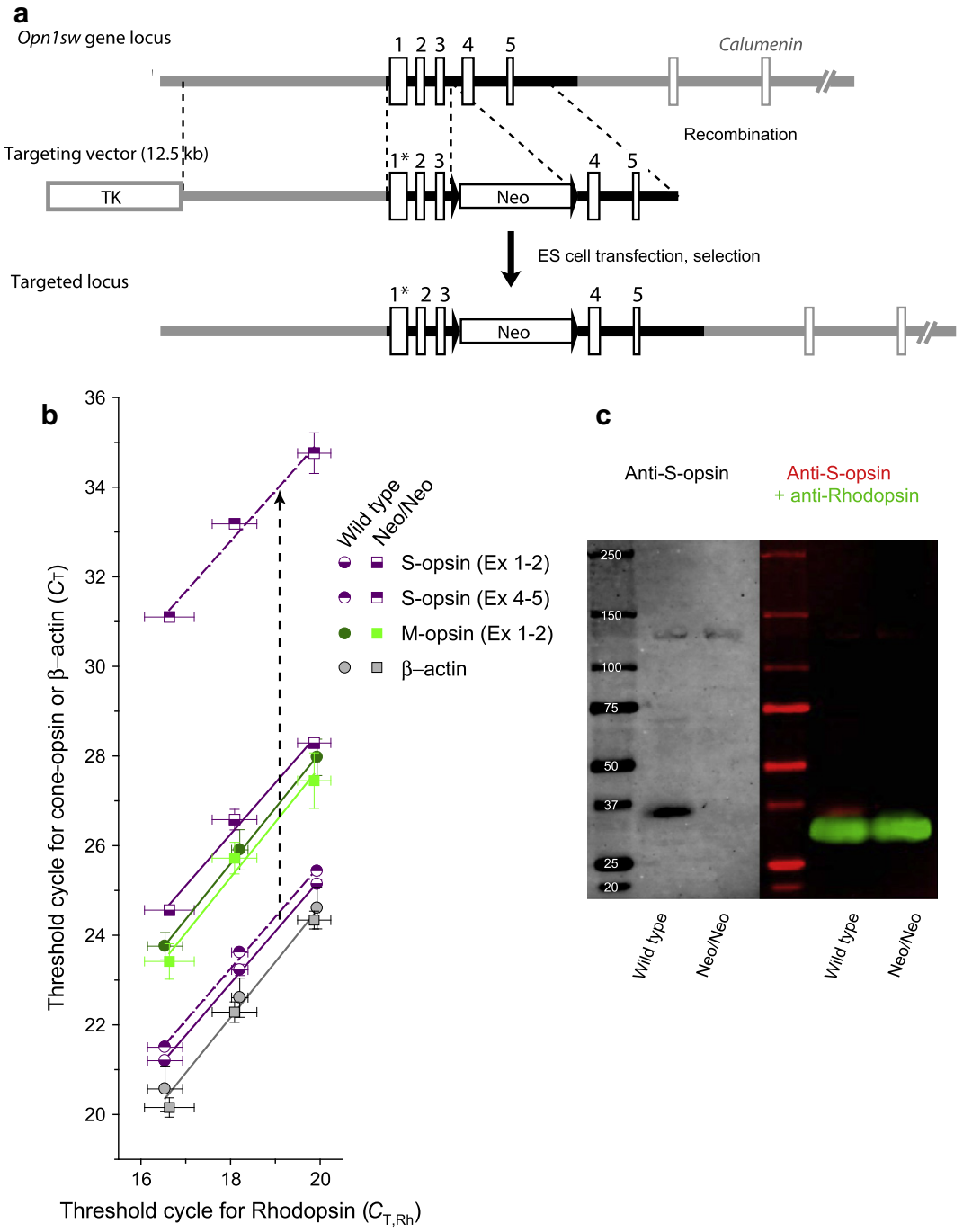

Fig. 1. S-opsin knock-in targeting strategy created a severely hypomorphic allele (*Opn1sw*^{Neo}): (a) *Schematic of Opn1sw targeting strategy*. The targeting vector comprised a Neomycin resistance cassette (Neo) flanked by FRT recombination sites (arrowheads) and homologous sequences (4.5 Kb 3′ and 816 bp 5′) to the *Opn1sw* gene locus (the calumenin gene, which abuts the 5′ end of the *Opn1sw* gene and is transcribed in the reverse direction on the complementary strand). The asterisk indicates the site of a targeted point mutation. Southern blotting and PCR confirmed successful targeting. (b) *Real-time quantitative PCR results*. The threshold cycle (C_T) of Taq-Man PCR reactions with primers for S-opsin (purple), M-opsin (green) and β-actin (gray) message are plotted against C_T for reactions with primers for rhodopsin message. Three dilutions of cDNA templates generated from *Opn1sw*$^{Neo/Neo}$ (squares) and WT littermate control (circles) served as the input to the PCR reactions. Primer sequences spanned exon junctions 1–2 (filled or bottom half-filled) or 4–5 (top half-filled) symbols. Data were obtained from mRNA extracted from the entire eyes of an *Opn1sw*$^{Neo/Neo}$ and a WT littermate control. Error bars are standard deviations: observations with 1× dilution of the cDNA from the reverse transcriptase reaction were replicated 2× for each data point, those with ¼ dilution 4× and those with 1/16 dilution 8×. The straight lines, fitted by least-squares to the data, are very nearly parallel (slopes varied by 10%, ranging from 1.13 to 1.24), so that the vertical offset of the lines representing the same transcript in *Opn1sw*$^{Neo/Neo}$ and WT retinas provide load-independent estimates of differences in the transcripts. (c) *Immunoblotting with an anti-S-opsin antibody detects no S-opsin in the Opn1sw*$^{Neo/Neo}$ *retina*. Extracts of WT and *Opn1sw*$^{Neo/Neo}$ retinas containing 60 pmol rhodopsin (corresponding to ~10% of the total retina) were loaded into adjacent gel lanes, and probed with antibodies for S-opsin (left panel, grayscale presentation), or for S-opsin (right panel, red) and rhodopsin (green). No S-opsin is detected in the *Opn1sw*$^{Neo/Neo}$ retina lane. Control experiments (Supplement, Fig. 1S) show that ~30 fmol S-opsin would be detectable.