Feedback regulation of cone cyclic nucleotide channels by phosphoinositides. Focus on “CNGA3 achromatopsia-associated mutation potentiates the phosphoinositide sensitivity of cone photoreceptor CNG channels by altering intersubunit interactions”

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THE TRANSFORMATION OF LIGHT into electrical signals is the first step in the visual response. The molecular machinery responsible for this transformation is arguably the best understood cellular signaling pathway. In this pathway, light-mediated activation of the G protein-coupled receptor rhodopsin (R) triggers the G protein transducin (T) to exchange GDP for GTP. One activated rhodopsin (R*) can activate hundreds of molecules of transducin; each transducin-GTP complex in turn activates phosphodiesterase type 6 (PDE6). PDE6 hydrolyzes cGMP to 5'-GMP, lowering cGMP to levels that no longer activate cyclic nucleotide-gated (CNG) channels. Closure of CNG channels disrupts the balance of ionic fluxes and hyperpolarizes the plasma membrane, thereby generating an electrical signal. Several feedback mechanisms are critical for rapid shutdown of this signal (2, 4): phosphorylation of R* leads to arrestin binding, receptor desensitization, and thus blocks R-mediated activation of T; RGS9 accelerates the GTPase activity of T, shutting down T-mediated activation of PDE6; and closure of CNG channels lowers intracellular Ca2+ levels and relieves Ca2+-mediated inhibition of guanylyl cyclase (GC) activity, increasing cGMP production, and a more rapid recovery to baseline cGMP levels. Both signal amplification and feedback regulation are critical in generating reproducible electrical signals in response to changes in light.

Feedback regulation also underlies adaptation to dim or bright backgrounds. Ca2+-mediated feedback is thought to be largely responsible for adaptation and involves several processes: Ca2+-mediated inhibition of GC activity; Ca2+-calmodulin-mediated inhibition of CNG channels—a small effect in cones; and, in cones, modulation of CNG channel activity by CNG-modulin (4, 7, 10). The potential roles of other messengers in feedback regulation of the light response are less well understood. Phosphoinositides (PIPs) are one such set of messengers (Fig. 1A). Photoreceptors contain the enzymes necessary for PIP turnover and have increased PIP production and degradation in response to sustained exposure to light (8). PIPs have been implicated both in the enhancement of transducin-PDE6 interactions leading to increased PDE6 activity and in the inhibition of cone CNG channels (3, 9). However, the roles of PIPs in regulating light responses have been difficult to discern, in part because of a limited understanding of the molecular mechanisms underlying PIP-mediated feedback and of the changes in PIP levels triggered by variations in background light levels. Recently, M. D. Varnum and colleagues have unraveled the mechanisms by which PIPs alter the cyclic nucleotide sensitivity of cone CNG channels (5, 6).

CNG channels are tetramers with four cyclic nucleotide binding sites and a common ion conduction pore. The binding

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**Fig. 1.** Depiction of phosphoinositide (PIP)-mediated regulation of cGMP signals in cone outer segments. A: PIPs potentiate transducin-mediated activation of PDE activity (green arrow) and inhibit cyclic nucleotide-gated (CNG) channels (CNGCs, red arrow). B: in cones containing CNG channels with the achromatopsia-associated mutation L633P (L633P-CNGC), it is anticipated that cGMP will more readily activate L633P-CNGCs when PIP levels are low and that cGMP will have reduced ability to activate channels when PIP levels are high. [Schematic adapted from Baylor (2).]
of cAMP or cGMP to one or more sites increases the time the protein spends in an open (ion-conducting) configuration. The apparent affinity of CNG channels for either cAMP or cGMP is altered by the binding of PIPs. Mutations to CNG channels are linked to several retinal channelopathies, including achromatopsia, a disease in which cone dysfunction leads to color blindness. Varnum and colleagues have taken advantage of the ability to probe CNG channel function in response to cyclic nucleotides and PIPs to unravel the impact of the achromatopsia-associated mutation L633P.

Cone CNG tetramers contain CNGA3 and CNGB3 subunits. Homotetramers of CNGA3 and CNGB3 subunits have altered sensitivity to cyclic nucleotides and PIPs. Recently, Dai et al. (5) presented data demonstrating that mutagenesis of two distinct, positively charged modules within the CNGA3 subunit prevent PIP-mediated regulation of CNGA3 homomultimeric and CNGA3/CNGB3 heteromultimeric channels. These results provided a framework with which to explore the effects of the L633P mutation on subunit interactions underlying PIP-mediated regulation of cone CNG channels.

In this issue of *American Journal of Physiology-Cell Physiology*, Dai and Varnum (6) take advantage of this framework and provide compelling evidence that intersubunit interactions mediate the regulation of cone CNG channels by PIPs. Specifically, Dai and Varnum investigated the mechanisms by which the achromatopsia-associated mutation L633P increases PIP-mediated inhibition of channel activity. L633 is located just past the COOH-terminal cyclic nucleotide-binding domain of the CNGA3 subunit, within the COOH-terminal PIP regulation module. The authors observed that 1) homomultimers composed of CNGA3 subunits with the L633P mutation (L633P-CNGA3) and heteromultimers of L633P-CNGA3 and CNGB3 subunits had increased apparent affinities for cGMP; 2) channels composed of L633P-CNGA3 subunits displayed blunted PIP-induced increases in cAMP mediated currents; 3) channels with the L633P mutation displayed potentiated PIP-mediated inhibition of cGMP-induced currents; and 4) neutralization of the positively charged residues in the NH2-terminal PIP-regulation module (preventing PIP-mediated inhibition of cGMP-induced currents) abolished augmentation of PIP-mediated inhibition by the L633P mutation. These observations led Dai and Varnum to hypothesize that direct NH2- and COOH-terminal interactions are responsible for PIP-mediated inhibition of CNG channels, and that the L633P mutation enhances these interactions. Consistent with their hypothesis, coimmunoprecipitation of a green fluorescent protein (GFP)-labeled COOH-terminal truncation mutant of CNGA3 and a GST-tagged COOH-terminal fragment demonstrated the potential for direct interactions between these domains, and tandem dimer approaches indicated that the interactions occur between subunits. These multifaceted approaches provide a persuasive argument for direct interactions between the NH2- and COOH-terminal regions of CNGA3.

The results presented by Dai and Varnum give insight into the molecular mechanisms of PIP-mediated regulation of cone CNG channels and potential effects of the achromatopsia-associated mutation L633P on CNG channel activity in cone outer segments (Fig. 1B). However, the roles of PIPs in regulating the light response have not yet been discerned because we lack a quantitative understanding of how sustained changes in background light levels, or other physiological stimuli, alter PIP levels. Current single-cell approaches for measurement of PIPs utilize GFP-labeled lipid recognition sequences to image changes in the levels of specific PIPs (1). While these approaches offer insight into changes in intracellular PIP levels, difficulties occur with their use in photoreceptors, including a lack of PIP specificity, the effects of prolonged changes in background illumination on fluorescence measurements, and effects of repeated fluorescent measurements on the light response. Utilizing current probes in carefully controlled experiments—or investing resources into development of second generation probes—may help lead to a quantitative understanding of PIP-mediated feedback in adaptation and to discovering how altering PIP-mediated feedback contributes to achromatopsia.

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AUTHOR CONTRIBUTIONS

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