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Probing functional interfaces of rod PDE γ-subunit using scanning fluorescent labeling

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Abstract

In the dark, the activity of the rod cGMP phosphodiesterase (PDE) catalytic α- and β-subunits (Pαβ) is inhibited by two γ-subunits (Pγ). On light stimulation of the photoreceptor cells, the GTP-bound α-subunit of visual G-protein transducin (G_{ta}GTP) displaces the Pγ-subunits from their inhibitory sites on Pαβ, leading to the effector enzyme activation. We designed a number of Pγ mutants, each with a single cysteine residue evenly distributed at a different position along the Pγ polypeptide chain. These cysteine residues served as sites for the introduction of the environmentally sensitive fluorescent probe, 3-(bromoacetyl)-7-diethyl aminocoumarin (BC). Analysis of the interactions of Pαβ and G_{ta} with the fluorescently labeled Pγ mutants suggests two distinct functional interfaces of Pγ. The Pαβ/Pγ interface is formed essentially by the C-terminus of Pγ and by the N-terminal portion of the Pγ polycationic region, Pγ-24-45, whereas the Pγ/G_{ta} interface includes the C-terminal portion of Pγ-24-45 and the region surrounding Pγ Cys68. Such functional organization of Pγ may represent an important element for the PDE activation mechanism during transduction of visual signals.