[2] Assays of G Protein/cGMP–Phosphodiesterase Interactions

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Introduction

Activation of cGMP phosphodiesterase (PDE) by the photoreceptor G protein, transducin (G_t), is a key event in the vertebrate visual transduction cascade. In the dark, the GDP-bound transducin α subunit ($G_t\alpha$) is complexed with $G_t\beta\gamma$. Photoexcitation of the visual receptor rhodopsin induces a GDP/GTP exchange on $G_t\alpha$ and dissociation of the GTP-bound $G_t\alpha$. $G_t\alpha$GTP rapidly activates PDE, leading to a drop in the intracellular concentration of cGMP in photoreceptor cells. The rod photoreceptor PDE is composed of two homologous catalytic subunits ($P\alpha\beta$) that are kept inactive in the dark via the association with two identical inhibitory γ subunits ($P\gamma$). $G_t\alpha$GTP binds to the $P\gamma$ subunits and displaces them from the $P\alpha\beta$ catalytic sites, thus producing an active enzyme.[1–3]

The assays of cGMP hydrolysis by activated PDE have been a major tool for monitoring the interaction between transducin and PDE, and initial studies mainly relied on such assays.[4,5] Purified $G_t\alpha$ complexed with the nonhydrolyzable analog of GTP, GTPγS, stimulates PDE in solution ineffectively, and the PDE activation assay requires relatively large concentrations of $G_t\alpha$.[5] The presence of rod outer segment (ROS) membranes or lipid vesicles significantly enhances the effectiveness of PDE stimulation because of the formation of an active membrane-bound complex between $G_t\alpha$ and PDE and allows an increase in assay sensitivity.[6,7] However, membrane-supported activation of PDE by transducin is a complex process that depends on a number of factors such as type and concentration of membranes (vesicles), binding of PDE, binding of $G_t\alpha$ to membranes, and the intact state of lipid modifications on $P\alpha\beta$ and $G_t\alpha$. Furthermore, PDE activation is not a direct monitor of transducin binding to the enzyme. This is evident from studies of $G_t\alpha$ mutants that bind but fail to activate PDE,[8] or interact with the effector weakly without causing enzyme activation.[9] Therefore, detailed analysis of the interface

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