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MEETING ABSTRACT Open Access

Asymmetric properties of rod cGMP Phosphodiesterase 6 (PDE6): structural and functional analysis

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Photoreceptor cGMP Phsophodiesterase 6 (PDE6) is the effector molecule of visual signal transduction and mediates fast response of light signals. The rod holo-PDE6 comprises catalytic (α, β; each ~ 90 kDa) and two identical inhibitiory (γ; ~ 10 kDa) subunits. The catalytic subunits comprise N-terminal tandem GAF domains followed by C-terminal catalytic domains and isoprenylations for membrane-association. Contrary to activation of other tandem GAF comprising PDEs, PDE6 activation does not occur via cGMP-induced concerted conformational changes. Rather two copies of the α-subunit of retinal G-Protein (Gα*), transducin, activate PDE6 by partially displacing the inhibitory subunits. The activation of PDE6 has therefore been described as a "de-inhibition". The affinity of Gα* to PDE6 and the enzymatic activity of the intermediary 1:1 complex is highly disputed, therefore a conclusive activation model is lacking so far.

Our combined structural, enzymatic and computational investigations deal with the activation-mechanism of PDE6. Our cryo electron microscopy (EM) structure of PDEαβ catalytic core shows an elongated bell-shaped structure with symmetric side-by-side arrangement of the two subunits with flexible membrane-binding domains. A comparison with nearly full-length inactive PDE2A structure [1] suggests that less compaction of both subunits and higher degree of conformational freedom of the catalytic domains result in constitutive activation of PDE6αβ, which is kept inactive by the inhibitory γ subunits. Furthermore, the structure of PDE6 suggests Gα* binding-sites pointing to opposing faces. The enzymatic characterization using Gα* titration of the PDE6 however reveal striking asymmetry of the two catalytic subunits with a high and a low affinity binding site for Gα*. Occupancy of the PDE6 with one Gα* induces negligible activity, whereas occupancy with two copies of Gα* leads to full enzyme activity. Such an activation mechanism constitutes a "coincidence switch" that allows noise filtering (i.e., spontaneously produced Gα* do not activate PDE6). Our spatiotemporal simulation work indeed confirms that spontaneously generated Gα* lead to the formation of singly occupied PDE6 and only a high local concentration of Gα*, as produced by an active receptor (rhodopsin), leads to doubly Gα* occupied effector complex. Therefore the localized large concentration of Gα* combined with the asymmetric properties of PDE6 constitutes a "density switch" that allows suppression effector level noise and reliable reporting of single quantum events in rod photoreceptor cells.

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