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Structure and dynamics of human cone phosphodiesterase 6

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1. Main Text

Photoreceptor phosphodiesterase 6 (PDE6) is a key effector in the visual excitation pathway in rod and cone photoreceptor cells. Failure of PDE6 to properly maturate into a functional enzyme causes severe retinal diseases. The maturation and activity of PDE6 is critically dependent on HSP90, its specialized co-chaperone aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1), and the regulatory Pγ subunit of PDE6. Our goal is to understand the molecular basis of cone PDE6 maturation and determine its structures in different conformational states.

2. Methods and results

Methods: EGFP-fused human cone PDE6C was expressed in the presence or absence of AIPL1 and/or Pγ using insect cell culture. Various preparations of PDE6C were purified by immunoaffinity chromatography over EGFP-nanobody resin followed by PDE6C release with 3C protease and size exclusion chromatography. The PDE6C samples were analyzed by single-particle cryo-EM.

Results: The structure of the nearly full-length (PDE6C)$_2$Pγ_2 complex was determined by single-particle cryo-EM analysis in the presence of exogeneous cGMP. The structure modeled into the final map of PDE6C at 3.0 A resolution featured the well-resolved GAF-A, GAF-B and catalytic domains (Fig. 1)[1]. The structure also showed the Pγ subunits bound to the catalytic sites and the GAF-B, but not the GAF-A domains. Interestingly, despite the occlusion of the catalytic opening by the C-terminus of Pγ, the catalytic pocket contained bound 5’-GMP. Variability analysis uncovered the dynamic nature of Pγ association with the PDE6C catalytic subunits. Cryo-EM analysis of holoPDE6C purified in the absence of exogeneous cGMP revealed two dominant conformations of PDE6C, one of which lacked the density for the GAF-A domains and apparently reflected dissociation of cGMP from the noncatalytic sites. Furthermore, we analysed the structure of PDE6C with Pγ removed by limited tryptic proteolysis, which revealed dramatic rearrangement of the PDE6C catalytic dimers. Overall, our analysis of human cone PDE6 by cryo-EM revealed the allosteric conformational changes and protein dynamics that are predicted to be essential for the enzyme maturation and function.

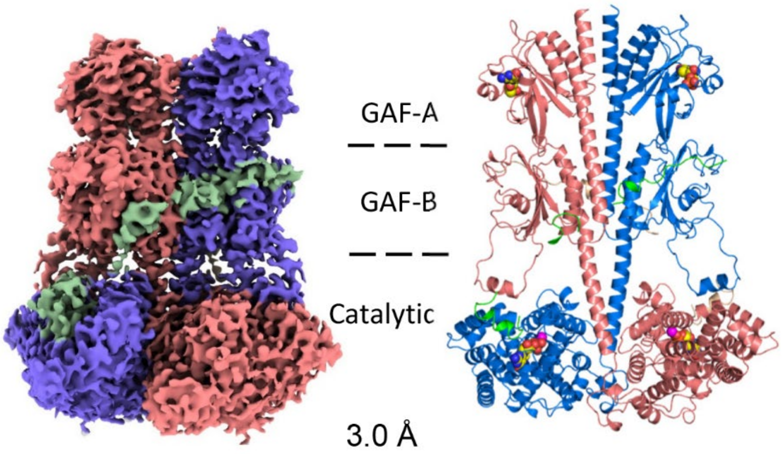

Fig. 1. Cryo-EM structure of human cone PDE6C

3. Acknowledgement

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4. References

[1] Singh S, Srivastava D, Boyd K, Artemyev NO. Structural and functional dynamics of human cone cGMP-phosphodiesterase important for photopic vision. Proc Natl Acad Sci U S A., 122(1), 2025

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