Title:

Structural and mechanistic basis for Gβγ-mediated activation of phosphoinositide 3-kinase γ

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Abstract

Phosphoinositide 3-kinase γ (PI3Kγ) converts PIP_2 to PIP_3 in a key step of leukocyte chemotaxis. However, it is also highly expressed in some cancers where it contributes to metastasis, particularly in prostate, breast, and pancreatic cancer. PI3Kg contains a catalytic subunit (p110γ) and a regulatory subunit (p101), and activation of PI3Kγ is coordinately regulated by Gβγ subunits and Ras, which are activated downstream of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), respectively. However, detailed molecular mechanisms underlying the Gβγ- and Ras-mediated activation of PI3Kγ remain unclear. In this study, we hypothesize that Gβγ binding triggers conformational changes of PI3Kγ, leading to its activation. To test the hypothesis, we purified functional PI3Kγ that demonstrates its Gβγ-dependent activation in the *in vitro* kinase assays. We then determined the cryo-EM structure of native PI3Kγ (3.0 Å resolution) and various cryo-EM reconstructions of the PI3Kγ–Gβγ complexes (3.5-3.6 Å resolution), revealing two distinct Gβγ binding sites, one on the helical domain of p110γ and one on the C-terminal domain of p101. Conformational changes have been observed upon Gβγ binding to PI3Kγ, and key residues that were identified can be associated to allosteric activation. *In vitro* kinase assays and rescue experiments in Zebrafish are consistent with these findings, which allow for functional investigation of Gβγ-mediated activation mechanisms and will potentially aid in future drug development targeting PI3Kγ for selective cancer therapeutics, based on its unique responsiveness to Gβγ among PI3K isozymes.