ESRF	**Experiment title:** Cambridge MRC Block Allocation Group Structure of a Ras/phosphoinositide 3-kinase complex	**Experiment number:** LS-1669
Beamline: ID14-2 ID14-1	**Date of experiment:** from: 5/3/00 to: 8/3/00 from 20/4/00 to: 22/4/00	**Date of report:** 28/8/00
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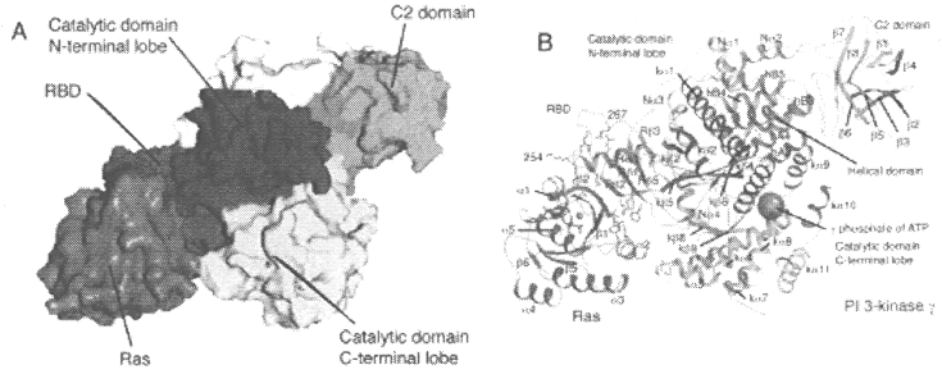
Report:

Ras activation of phosphoinositide 3-kinase (PI3K) is important for survival of transformed cells. We find that PI3Kγ is strongly and directly activated by H-Ras G12V *in vivo* or by GTPγS-loaded H-Ras *in vitro*. We have determined a crystal structure of a PI3Kγ/Ras•GMPPNP complex. A critical loop in the Ras-binding domain positions Ras so that it uses its switch I and switch II regions to bind PI3Kγ. Mutagenesis shows that interactions with both regions are essential for binding PI3Kγ. Remarkably, Ras also forms a direct contact with the PI3K catalytic domain. These unique Ras/PI3Kγ interactions are likely to be shared by PI3Kα. The complex with Ras shows a change in the PI3K conformation that may represent an allosteric component of Ras activation.

A data set was collected to 3.0 Å resolution using two PI3K/Ras co-crystals. Becaause crystals diffracting beyond 3.0 Å were fairly uncommon, approximately 40 crystals were screened. The structure

was solved by molecular replacement using our previously-determined structure of human PI 3-kinase (Walker *et al.*, 1999; Walker *et al.* 2000) and the structure of Ras taken from the Ras/GalGDS complex (pdb entry 1LFD). The manuscript describing the structure and activation of PI3Kγ by Ras has been submitted (Pacold *et al*).

(A) Solvent-accessible surface of the Ras•PI3Kγ complex. The Ras (orange) and four domains of the PI3Kγ, comprising the RBD (purple), C2 domain (cyan), helical domain (green) and N and C-terminal lobes of the catalytic domain (red and yellow) are shown. The N-terminal linker is rendered in white. (B) Ribbon diagram of the Ras•PI3Kγ complex. The color scheme is the same as the previous panel. The location of the γ phosphate of ATP/PI3Kγ structure is marked with a large gray sphere. This location roughly corresponds to the phosphoinositide headgroup binding site.



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Pacold, M.E., Suire, S., Perisic, O., Lara-Gonzalez, S., Davis, C.T., Walker, E.H., Hawkins, P.T., Stephens, L., Eccleston, J.F., Williams, R.L. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase γ. *Submitted.*