Quantitation of class IA PI3Ks in mice reveals p110-free-p85s and isoform-selective subunit associations and recruitment to receptors

N. Tsolakos1,2, T. N. Durrant1, T. Chessa1,2, S. M. Suire3, D. Oxley4, S. Kulkarni4, J. Downward4, O. Pericis5, R. L. Williams6, L. Stephens1,2,3, and P. T. Hawkins2,3

The Signaling Department, The Babraham Institute, CB22 3AT Cambridge, United Kingdom; Department of Chemistry, University of Oxford, OX1 3QZ Oxford, United Kingdom; The Mass Spec Facility, The Babraham Institute, CB22 3AT Cambridge, United Kingdom; The Oncogene Biology Lab, The Francis Crick Institute, NW1 1AT London, United Kingdom; and 6Protein and Nucleic Acid Chemistry, MRC Laboratory of Molecular Biology, CB2 0QH Cambridge, United Kingdom

Edited by Kevan M. Shokat, University of California, San Francisco, CA, and approved October 11, 2018 (received for review March 1, 2018)

Class IA PI3Ks have many roles in health and disease. The rules that govern intersubunit and receptor associations, however, remain unclear. We engineered mouse lines in which individual endogenous class IA PI3K subunits were C-terminally tagged with 17aa that could be biotinylated in vivo. Using these tools we quantified PI3K subunits in streptavidin or PDGFR pull-downs and cell lysates. This revealed that p85α and β bound equivalently to p110α or p110β but p85α bound preferentially to p110α. p85α were found in molar-excess over p110α in a number of contexts including MEFs (p85p55, 20%) and liver (p85α, 30%). In serum-starved MEFS, p110-free-p85p55 were preferentially, compared with heterodimeric p85, bound to PDGFRs, consistent with in vitro assays that demonstrated they bound PDGFR-based tyrosine-phosphorylated peptides with higher affinity and cooperativity; suggesting they may act to tune a PI3K activation threshold. p110α-heterodimers were recruited 5-6x more efficiently than p110β-heterodimers to activated PDGFRs in MEFs or to PDGFR-based tyrosine-phosphorylated peptides in MEFS-lysatess. This suggests that PI3Kα has a higher affinity for relevant tyrosine-phosphorylated motifs than PI3Kβ. Nevertheless, PI3Kβ contributes substantially to acute PDGFR-stimulation of PIP3, and PKB in MEFS because it is synergistically, and possibly sequentially, activated by receptor-recruitment and small GTPases (Rac/CDC42) via its RBD, whereas parallel activation of PI3Kα is independent of its RBD. These results begin to provide molecular clarity to the rules of engagement between class IA PI3K subunits in vivo and past work describing “excess p85,” p85α as a tumor suppressor, and differential receptor activation of PI3Kα and PI3Kβ.

PI3K | Avi-tag | isoform-selective

Class IA PI3Ks are heterodimers with a regulatory (p85α, p85β, or p55γ) and a catalytic subunit (p110α, β, or δ, which give their name to a complex). They make the signaling lipid PIP3, that is sensed by a range of effectors [e.g., protein kinase B (PKB), Bruton’s tyrosine kinase (BTK), PI3P-dependent Rac exchange factor 1 (PReX1), PReX1] that control metabolism, the cytoskeleton, and growth. Genetic alterations that augment the activity of these subunits allow selective activation of PI3Kα and β by receptors.

Author contributions: L.S. and P.T.H. designed research; N.T., T.N.D., T.C., S.M.S., and D.O. performed research; S.K., J.D., O.P., and R.L.W. contributed new reagents/analytic tools; N.T., T.N.D., T.C., S.M.S., D.O., L.S., and P.T.H. analyzed data; and N.T., T.N.D., T.C., S.M.S., R.L.W., L.S., and P.T.H. wrote the paper. The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1N.T., T.N.D., and T.C. contributed equally to this work.

2L.S. and P.T.H. contributed equally to this work.

3To whom correspondence may be addressed. Email: len.stephens@babraham.ac.uk or philip.hawkins@babraham.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1803446115/-/DCSupplemental.

Published online November 15, 2018.
because of differences in the properties and/or concentrations of p110α. However, this relies on the dogmas that there is little intersubunit selectivity and/or that p85α do not introduce specificity. The first of these assumptions has not been tested directly and the latter is contradicted by reports of differences in the properties of p85α and p85β (4, 5, 15, 16).

The concept of p110-free and p110-independent functions of p85α have been raised many times, including a recent report that PIK3RI (encoding p85α) can be a tumor suppressor (17–22) and evidence that p110-free p85α are targeted for isoform-specific degradation (18, 23). Some work has provided evidence for specific p110-free regulatory subunit complexes (19); however, the best quantitative analysis of class IA PI3K subunit stoichiometry concluded there were no p110-free regulatory subunits (24). We have addressed these questions.

Results

We used standard homologous targeting technology in mouse ES cells to derive mouse strains expressing; either the biotin ligase BirA [the prokaryotic biotin ligase BirA modified to have mammalian codon usage (25)] from the endogenous ROSA26 locus (mBirA+/−) or endogenous, C-terminal avi-tagged [17a, containing a 15aa minimal consensus for BirA (26)] p85α, p85β, p110α, p110β, or p110δ (e.g., p85α avi/avi), which will tag all three splice-variants of p85α, all in a C57BL/6J background (SI Appendix, Figs. S1–S7). The mice were interbred to express mBirA or the avi-tagged alleles alone or together (e.g., mBirA+/−, p85α avi/avi x mBirA+/−). All of these strains appeared healthy and fertile except p110α avi/avi—mice that did not reach adulthood. The majority died just before birth [p110α KD/KD mice die near E2 (27)]. It seems likely, on the basis of the results below, that this problem is due to the C-terminal avi-tag reducing, but not abating, the kinase activity of p110α; a phenomenon that has been reported before for other C-terminal tags (28). We derived MEFs from mice expressing each of the avi-tagged subunits and introduced mBirA (or not) and EGFP via viral transduction and sorted EGFP-positive cells for experiments (Fig. L1).

Tissue or cell lysates were prepared and subjected to streptavidin-directed pull-down under mild, nondenaturing conditions. Aliquots of the lysates before and after pull-down were immunoblotted with antibodies recognizing the avi-tag, p85α, p110α, p110β, or p110δ (e.g., p85α avi/avi) which will tag all three splice-variants of p85α, all in a C57BL/6J background (SI Appendix, Figs. S1–S7). The mice were interbred to express mBirA or the avi-tagged alleles alone or together (e.g., mBirA+/−, p85α avi/avi x mBirA+/−). All of these strains appeared healthy and fertile except p110α avi/avi—mice that did not reach adulthood. The majority died just before birth [p110α KD/KD mice die near E2 (27)]. It seems likely, on the basis of the results below, that this problem is due to the C-terminal avi-tag reducing, but not abating, the kinase activity of p110α; a phenomenon that has been reported before for other C-terminal tags (28). We derived MEFs from mice expressing each of the avi-tagged subunits and introduced mBirA (or not) and EGFP via viral transduction and sorted EGFP-positive cells for experiments (Fig. L1).

Tissue or cell lysates were prepared and subjected to streptavidin-directed pull-down under mild, nondenaturing conditions. Aliquots of the lysates before and after pull-down were immunoblotted with antibodies recognizing the avi-tag, p85α, p110α, p110β, or p110δ (e.g., p85α avi/avi) which will tag all three splice-variants of p85α, all in a C57BL/6J background (SI Appendix, Figs. S1–S7). The mice were interbred to express mBirA or the avi-tagged alleles alone or together (e.g., mBirA+/−, p85α avi/avi x mBirA+/−). All of these strains appeared healthy and fertile except p110α avi/avi—mice that did not reach adulthood. The majority died just before birth [p110α KD/KD mice die near E2 (27)]. It seems likely, on the basis of the results below, that this problem is due to the C-terminal avi-tag reducing, but not abating, the kinase activity of p110α; a phenomenon that has been reported before for other C-terminal tags (28). We derived MEFs from mice expressing each of the avi-tagged subunits and introduced mBirA (or not) and EGFP via viral transduction and sorted EGFP-positive cells for experiments (Fig. L1).

As the expression of the PI3K subunits was unchanged by avi-tagging, it seemed unlikely their turnover had been perturbed. This was confirmed in “chase” experiments in the presence of an inhibitor of protein synthesis (emetine) with p85α–MEFs that revealed the rate of degradation of endogenous avi-tagged p85α was indistinguishable from wild-type-p85α and Fig. 2; see also SI Appendix, Figs. S3–S7). The avi-tagged and/or biotinylated constructs resolved from the endogenous p85αs. Firstly, this made it clear, by internal comparison of wild-type and avi-tagged proteins in avi-heterozygous cells/tissues (e.g., p85α-immunoblots of p85α-avi-expressing MEFS, Fig. L4) or in avi-homozygous cells/tissues (e.g., p110δ-avi-expressing mouse tissues, Fig. 2) that the presence of the avi-tag had no effect on expression of any of the class IA PI3K subunits (for p85α and p110δ blot, see SI Appendix, Figs. S4 and S7).

Secondly, this made it possible to detect complete streptavidin-directed pull-down of avi-tagged, but not wild-type, proteins in the presence, but not the absence, of mBirA (Fig. L4 and B). These latter results demonstrate near 100%, mBirA- and avi-tag-dependent biotinylation and then streptavidin-mediated pull-down.

As the expression of the PI3K subunits was unchanged by avi-tagging, it seemed unlikely their turnover had been perturbed. This was confirmed in “chase” experiments in the presence of an inhibitor of protein synthesis (emetine) with p85α–MEFs that revealed the rate of degradation of endogenous avi-tagged p85α was indistinguishable from wild-type-p85α and Fig. 2; see also SI Appendix, Figs. S3–S7). The avi-tagged and/or biotinylated constructs resolved from the endogenous p85αs. Firstly, this made it clear, by internal comparison of wild-type and avi-tagged proteins in avi-heterozygous cells/tissues (e.g., p85α-immunoblots of p85α-avi-expressing MEFS, Fig. L4) or in avi-homozygous cells/tissues (e.g., p110δ-avi-expressing mouse tissues, Fig. 2) that the presence of the avi-tag had no effect on expression of any of the class IA PI3K subunits (for p85α and p110δ blot, see SI Appendix, Figs. S4 and S7).

Secondly, this made it possible to detect complete streptavidin-directed pull-down of avi-tagged, but not wild-type, proteins in the presence, but not the absence, of mBirA (Fig. L4 and B). These latter results demonstrate near 100%, mBirA- and avi-tag-dependent biotinylation and then streptavidin-mediated pull-down.

Quantification of anti-avi immunoblots revealed that p85α and p110β are the major isoforms of the regulatory and catalytic subunits in MEFS (Fig. 1C), confirming earlier work (24). We also quantified the expression of the individual avi-tagged subunits in cell lines expressing the relevant avi-tagged proteins by streptavidin pull-down, clution, and mass spectrometry in the presence of multiple, internal, heavy-labeled, synthetic peptide standards to correct for recovery (Fig. 1D and SI Appendix, Figs. S9–S11). This confirmed the results with anti-avi immunoblots and indicated the levels of p85α (almost exclusively the largest variant) and p110β to be 58,000 or 42,000 molecules/cell, respectively (intracellular concentrations of 32 or 23 nM). Deeper analysis of these pull-downs (using the heavy-peptide standards) allowed us to quantify all of the other class IA PI3K subunits that were recovered in the pull-downs and hence obtain two (from the p85α avi/avi and p110δ avi/avi pull-downs) independent, direct estimates for the absolute concentration of each heterodimer (Fig. 1E and SI Appendix, Fig. S11A). The relative concentrations of heterodimers were: p85α/p110β > p85α/p110α = p85β/p110α = p85β/p110β >> p85α/p110δ & p85β/p110δ. Very low levels of p55γ were recovered with avi-tagged p110α or β, in keeping with...
Fig. 3. p110-free p85 is present in MEFs and certain murine tissues. (A) The absolute amounts of avi-tagged p85s compared with the amount of p110s recovered with them in streptavidin pull-downs from p85α<sup>Avi/Avi</sup> or p85β<sup>Avi/Avi</sup> MEFs, corrected for input protein (means ± SD, from two clones/genotype, each clone was measured three times). (B) and (C) Experiments like those in (A), except using tissues from mice expressing avi-tagged p85s and mBirA. Data are corrected for input protein (means ± 5D, from three independent mice). (D) The percentage of total p110-free p85 in different mouse tissues was calculated from results shown in A–C. Statistically significant at *P < 0.05, **P < 0.01, and ***P < 0.001. NS, not significant. The underlying data are in SI Appendix, Fig. S11.

Fig. 4. p85α and p110β, but not other subunits, interact preferentially in vivo. (A) Compares the expected (e) and the measured (m), by streptavidin pull-down and mass spec, levels of the different class IA PI3K heterodimers in MEFs. Expected levels were calculated based on total p85 levels in the respective MEFs (SI Appendix, Fig. S12) accounting for the percentage of p85α or p110β bound to p110 (Fig. 3) expressed as percentages of the total p85. Data are means ± SD, from two MEF clones/genotype. (B) The levels of p110 subunits bound to p85α or p110β in the bone marrow or spleen of mice expressing relevant avi-tagged PI3K constructs. The levels are expressed as percentages of the total p110 measured by mass spec. Data shown are means ± SD, from three mice. (C) The levels of p110 in lysates of spleens from mice expressing mBirA and either avi-tagged p85α or p110β, before (pre) and after (post) streptavidin pull-down, as measured by immunoblotting. Parallel experiments for p85β and ICOP, that control for the efficiency of pull-down and cell-input, are also shown. The data are from a typical experiment repeated twice. Statistically significant at *P < 0.05, **P < 0.01, and ***P < 0.001. NS, not significant.
Quantitative analyses of class IA PI3K subunits in PDGFR-IPs from MEFs also found evidence for p110-free p85 (Fig. 6 A–C and SI Appendix, Fig. S14). Because of the context of these assays, we could only measure the total, PDGFR-associated p110-free p85 but not the proportions that were p85αω or p85β. Significantly, PDGFRs were enriched in p110-free p85, compared with heterodimeric p85, under basal unstimulated conditions and in the presence of low concentrations of PDGF (Fig. 6 A and B). Consistent with this, the proportion of p110-free p85p85αω that could be recovered by streptavidin from lysates of p85αω/p85αω, expressing MEFs was reduced by prior IP of PDGFRs (Fig. 6 C). These results suggest that p110-free p85 has a higher affinity than heterodimeric-p85 for tyrosine-phosphorylated PDGFRs. This hypothesis was confirmed by in vitro binding experiments (Fig. 6 D) which showed that p85αω bound PYPs with higher affinity and co-operativity than p85αω/p85αω.

PDG stimulates a transient accumulation of PI3P leading to phosphorylation of PKB in MEFs (7, 29). Activation of PKB has been shown to be substantially reduced, at lower doses of PDGF, in p110αω−/−, but not p110β−/−, MEFs (29, 30). According to our results suggesting P13Kα and β are both, although differentially, recruited to PDGFRs, we determined their roles in PDGF-stimulated PI3P accumulation in MEFs. BYL-719 inhibited PI3P accumulation (Fig. 7 A) by about 40%. This inhibition was manifest across a range of PDGF concentrations (Fig. 7 B) and further increased by a submaximal inhibitory dose of PI3Kα-selective inhibitor (Fig. 7 A). Interestingly, although TGF-β21 inhibited PDGF-stimulated PKB phosphorylation, it did so only at lower concentrations of PDGF (Fig. 7 C), consistent with previous data (30) and the idea that PKB phosphorylation will be maximally activated by submaximal amounts of PI3P. These results suggest that P13Kα and P13Kβ are both activated by PDGFRs and that they have both unique and overlapping roles in PI3P accumulation.

The quantitatively similar roles of P13Kα and P13Kβ in PI3P accumulation contrasted with the preferential recruitment of P13Kα to PDGFRs. To understand if this was a result of a difference in their regulation by small GTPases, we obtained MEFs from mice expressing small-GTPase-insensitive, point-mutant knock-ins of p110αω and p110βω [Ras-insensitive-p110αω, p110αωT208D, K227A/T208D, K227A (7) and Rac/CDC42-insensitive-p110βω, p110βωS205D, K224A/S205D, K224A (6)]. Mice, and MEFs derived from them, expressing these constructs have been used to reveal important roles for the RBDs of P13Kα and β in tumorigenesis and some G protein-coupled receptor (GPCR) signaling via class I PI3Ks (6, 7). We measured PDGF-stimulated PI3P accumulation in these MEF lines and the association, of both the wild-type and small-GTPase-insensitive versions, of p110αω and p110βω with PDGFRs. We found that the RBD of p110αω was not needed for PDGF-stimulated PI3P accumulation (Fig. 8 A), consistent with previous work measuring PKB phosphorylation (7). In contrast, the RBD of p110βω was required for maximal PDGF-stimulated PI3P accumulation (Fig. 8 B and C; work indicating the RBD of p110βω is not required for PDGF-stimulated AKT phosphorylation (6) used 5 min stimulations and is entirely consistent with our results showing the role of the RBD is reduced at later times, Fig. 8 D). A comparison of the effect of a P13Kβ-selective inhibited PI3P accumulation (Fig. 7 A and B) by about 40%. This inhibition was manifest across a range of PDGF concentrations (Fig. 7 B) and further increased by a submaximal inhibitory dose of PI3Kα-selective inhibitor (Fig. 7 A). Interestingly, although TGF-β21 inhibited PDGF-stimulated PKB phosphorylation, it did so only at lower concentrations of PDGF (Fig. 7 C), consistent with previous data (30) and the idea that PKB phosphorylation can be maximally activated by submaximal amounts of PI3P. These results suggest that P13Kα and P13Kβ are both activated by PDGFRs and that they have both unique and overlapping roles in PI3P accumulation.
Despite a relatively small amount of PI3K that accumulates in MEFs expressing p85 and p85 proteins over at PDGFRs. The simplest explanation for these accumulation, it had no role in contribute very similar PDGFR-

inhibitor on PDGF-stimulated PIP3 accumulation in MEFs expressing either wild-type- or Rac/CDC42-insensitive versions of p110, suggested that p110 is substantially dependent on its RBD in this context (Fig. 8C).

To understand the role of the RBD in p110α and p110β association with, and activation by, PDGFRs we measured the recovery of these constructs with PDGFRs by mass spectrometry. We found that the ligand-stimulated association of p110α with PDGFRs was not dependent on its RBD’s ability to bind to Ras (Fig. 8D and SI Appendix, Fig. S15). These results confirm previous work indicating neither growth factor-stimulated PKB phosphorylation nor association of p85s with tyrosine-phosphorylated proteins are dependent on Ras binding to the RBD of p110α (7). Surprisingly, the results also showed that despite the very clear role for the RBD of p110β in PDGF-stimulated PIP3 accumulation, it had no role in binding of PI3Kβ to PDGFRs. The simplest explanation for these results is that Ras does not contribute to activation of PI3Kα at PDGFRs, whereas binding of active Rac/CDC42 is absolutely required for stimulation, but not binding, of PI3Kβ at PDGFRs.

Discussion

Our results show that p85α and p85β bind p110α or p110β with indistinguishable affinity in vivo and hence the concentrations of the heterodimers they can form are solely a function of the relative levels of the subunits. Interestingly, p110β preferentially complexes with p85α compared with p85β. These results suggest that PI3Kδ is hard-wired to use p85α- or avoid p85β-specific properties or functions; e.g., phosphorylation (15) or p85β-directed (18) or 85α-directed (23) ubiquitination and degradation. It is noteworthy that the phenotypes of mice lacking p85α or expressing kinase-dead p110β are most similar in B lymphocytes where PI3Kδ is dominant (31, 32). Hence our data resolve an important long-standing question about the rules of engagement in class IA PI3K signaling (9).

We find that p85α and p85β contribute very similar PDGFR-binding capabilities to class IA PI3Ks. This is consistent with the known similarities in phospho-peptide-binding properties of the N and C-terminal SH2 domains within and between class IA PI3K regulatory proteins (3). Reported differences in the properties of p85α and p85β, in different contexts (4, 5), are presumably due to other interactions and/or domains.

Our data, derived from analysis of both p85- and PDGFR-pull-downs, suggest that p110-free p85α and p85β are present in mouse tissues and cells. This provides direct, independent support for recent work identifying roles for p110-free p85s (19, 21, 22); p110-free p85s have higher affinity and more co-operative binding to tyrosine-phosphorylated proteins than heterodimeric p85 proteins. This property could allow relatively small amounts of p110-free p85s to compete effectively with heterodimeric PI3Ks for low-abundance phosphorylated YXXM motifs. This may explain their relative enrichment on PDGFRs in basal or weakly stimulated cells and suggests they may have a role in reducing basal RTK signaling noise and/or in setting a threshold level of receptor activation that must be achieved to drive class IA PI3K activation. The stoichiometry of p85s to potential phospho-tyrosine binding sites will be an important determinant of the impact of p110-free p85s on PI3K signaling. A large excess of tyrosine-phosphorylated “YXXM” proteins over p85s would suggest that the window in which p110-free p85 might act as an inhibitor would be very limited. The stoichiometry with which tyrosine residues in endogenous proteins are phosphorylated is difficult to measure. However, given that a number of YXXM-containing signaling proteins seem to have similar copies per cell as p85s [PDGFRs, EGF receptors, insulin receptor substrates (IRSs) in MEFs are all in the range 1 × 102-2 × 102 compared with 105 p85s] and maximal PDGFR activation leads to over 80% of total p85s being recruited with anti-PDGFR-IPs (Fig. 5 B and C), it seems that upon intense challenge there will be an excess of phosphorylated YXXM motifs and depletion of cytosolic p85s. Hence, p110-free
p85 could act to tune the threshold, but not the maximal, activation of class IA PI3K signaling. Although PI3Kα played the major role in PDGF-stimulated PIP3 synthesis, PI3Kβ generated a disproportionately larger amount than predicted by its expression in association with PDGF receptors. We speculated this might be a result of a difference in other signals integrated by PI3Kα and PI3Kβ. Our data show that binding of active Ras to the RBD of p110α is not needed for PDGF-stimulated association of PI3Kα with PDGF receptors or for pIP3 accumulation. In contrast, the equivalent binding of Ras/CDC42 to the RBD of p110β is required for PDGF-stimulated PI3Kβ-dependent pIP3 accumulation but not for association of PI3Kβ with PDGF receptors. The simplest explanation of the latter results is that, in vivo, PI3Kβ is extremely dependent on combined activation by Ras/CDC42 and the activated PDGF receptor, in line with a previous hypothesis (33). The two inputs to PI3Kβ could be engaged in any order. The most parsimonious explanation of our data is that they act sequentially, because of a requirement for PI3Kβ to be associated with PDGF receptors to become sensitive/accessible to Ras/CDC42 (34). In this model, the role of Ras/CDC42 might be to allow PDGF-associated PI3Kβ access to PI(4,5)P2.

Our results resolve some long-standing questions and also raise further questions regarding the origin of the selective interaction between p85α and p110α and the physiological purpose of p110- and p85-specific PDGF-BB signaling. It will also be important to extend our studies describing preferential recruitment of PI3Kβ versus PI3Kα to tandem phospho-tyrosines in PDGF receptors to other contexts involving different local sequences and numbers of phospho-tyrosine binding sites.

Materials and Methods

Generation of PI3Kα-Avi-Tag and mBiA Mice. Pik3ca-avi, Pik3cb-avi, Pik3cd-avi, Pik3r1-avi, Pik3r2-avi, and Rosa26-avi targeting vectors were generated by a combination of cloning, recombineering, and gene synthesis and used to generate targeted avitagged PI3K subunits homozygotes (except live p85α targeting with fluorescent 2α was quantified by immunoblotting with fluorescent 2α antibodies as described in the SI Appendix. Matters only Described in the SI Appendix. Antibodies and Reagents. Immuno blotting. Streptavidin-antibody-mediated pull-down. Sample preparation and analysis by mass spectrometry and absolute protein quantitation. Preparation of recombinant PI3Ks. Competition Assays with PYPs. Statistics.

ACKNOWLEDGMENTS. This work was supported by grants from the Bio technology and Biological Sciences Research Council (BBSRC) [BB/I004456/1 (to L.S. and P.T.H.) all Babraham Institute facilities are supported by a Core Capability Grant], Wellcome Trust [WT085889MA (to L.S. and P.T.H.) and National Cancer Research Institute [MR/R000409/1 (to T.C., L.S., and P.T.H.) all Babraham Institute facilities are supported by a Core Capability Grant]. We thank Dr. U. Mechold and Prof. V. Ogryzko for mBiAR cDNA and Dr. W. Dean for ZP3-Cre mice.

1. Torpe LM, Yuzugullu H, Zhao J (2015) PI3K in cancer: Divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 15:7–24.
2. Vanhaesebroeck B, et al. (1997) P110delta, a novel phosphoinositide 3-kinase in cells. Proc Natl Acad Sci USA 94:4330–4335.
3. Songyang Z, et al. (1993) SH2 domains recognize specific phosphopeptide sequences. Cell 72:677–778.
4. Hartley D, Meisner H, Corvera S (1995) Specific association of the beta isoform of the p110alpha phosphoinositide 3-kinase with the proto-oncogene c-erbB. J Biol Chem 270:18260–18263.
5. Alcazar I, et al. (2009) P85beta phosphoinositide 3-kinase regulates CD28 coreceptor function. Blood 113:3198–3208.
6. Fritsch R, et al. (2007) p110delta and RHOM families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. Cell 153:1050–1063.
7. Gupta S, et al. (2007) Binding of ras to phosphoinositide 3-kinase p110alpha is regulated by inter-subunit interactions and by p85 associates with phosphoinositide 3-kinase isoforms. J Biol Chem 282:18260–18263.
8. Burke JS, Williams RL (2013) Dynamic steps in receptor tyrosine kinase mediated activation of class IA phosphoinositide 3-kinases (PI3K) captured by HDX exchange (HDX-MS). Adv Biol Regul 53:97–110.
9. Backer JM (2010) The regulation of class IA PI3-kinases by inter-subunit interactions. Curr Top Microbiol Immunol 346:87–114.
10. Zhang X, et al. (2011) Structure of lipid kinase p110beta in complex with ATP and its substrate, PIP3, determines allosteric activation of class I PI3K. Mol Cell 44:547–558.
11. Foukas LC, et al. (2006) Critical role for the p110alpha phosphoinositide-3-OH kinase in p110alpha-deficient murine PDGFR (PYP, residues 735–7814) was expressed in Sf9 cells, purified, and various amounts were incubated with biotin-labeled doubly phosphorylated PYP, pulled down with streptavidin beads, and the associated p85α was quantified by immunoblots using fluorescent 2α antibodies as described in the SI Appendix.
12. Kuchay S, et al. (2013) FBXL2- and PTPL1-mediated degradation of p110-PI regulatory subunit controls the PI(3,4,5)P3 signaling cascade. Nat Cell Biol 15:472–480.
13. Winnay JN, Boucher J, Mori MA, Ueki K, Kahn CR (2010) A regulatory subunit of phosphoinositide 3-kinase increases the nuclear accumulation of X-box-binding protein-1 to modulate the unfolded protein response. Nat Med 16:438–445.
14. Charper RB, et al. (2010) Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. Proc Natl Acad Sci USA 107:5471–5476.
15. Cheneg LW, et al. (2015) Regulation of the PI3K pathway through a p85α monomer-homodimer equilibrium. eLife 4:e08666.
16. Thorpe LM, et al. (2017) PI3K–p110α mediates the oncogenic activity induced by loss of the novel tumor suppressor PI3Kβps5. Proc Natl Acad Sci USA 114:7095–7100.
17. Zhao JJ, et al. (2016) Insulin resistance and diabetes caused by genetic or diet-induced KBTBD2 deficiency in mice. Proc Natl Acad Sci USA 113:E6418–E6426.
18. Geering B, Cutillas PR, Nock G, Gharbi SI, Vanhaesebroeck B (2007) Class IA phosphoinositide 3-kinases are obligate p85-510 heterodimers. Proc Natl Acad Sci USA 104:7809–7814.
19. Mechold U, Gilbert C, Ogryzko V (2005) Caden optimization of the BirA enzyme gene mimics and enhances dynamic events in the natural activation of phosphoinositide 3-kinase. Mol Cell Biol 25:3666–3676.
20. Reif K, Gout I, Waterfield MD, Cantrell DA (1993) Divergent regulation of phosphatidylinositol 3-kinase-dependent signaling pathways by three different protein kinases of the p85alpha regulatory subunit. Mol Cell Biol 20:8035–8046.
21. Mechold U, Gilbert C, Ogryzko V (2005) Caden optimization of the BirA enzyme gene mimics and enhances dynamic events in the natural activation of phosphoinositide 3-kinase. Mol Cell Biol 25:3666–3676.
22. Burke JE, Williams RL (1999) A minimal peptide substrate in biotin holo enzyme synthetase-catalyzed biotinylation. Protein Sci 8:921–929.
23. Foukas LC, et al. (2006) Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. Nature 441:366–370.
24. Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC (2005) Signalling by PI3K p110alpha isoforms: Insights from gene-targeted mice. Trends Biochem Sci 30:194–204.
25. Burke JE, Williams RL (2015) Synergy in activating class I PI3Ks. J Biol Chem 290:88–98.
26. Beckett D, Kovaleva E, Schatz PJ (1999) A minimal peptide substrate in biotin holo enzyme synthetase-catalyzed biotinylation. Protein Sci 8:921–929.