Phosphoinositide 3-kinases (PI3K) are enzymes that transfer phosphate to position 3 of the phosphoinositide ring, regulating a variety of cell responses including survival, division, and transformation. PI3Ks are divided into three subclasses based on their primary structure and substrate specificity, but only the class I enzymes generate phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate (3-PtdIns) products in vivo. Basal levels of these lipids are very low in quiescent cells but increase rapidly and transiently following growth factor receptor (GFR) stimulation (for a review, see Refs. 1–4). 3-Poly-PtdIns recruits pleckstrin homology domain-containing proteins such as phosphoinositide-dependent protein kinase 1 and protein kinase B (PKB), which mediate PI3K signal propagation (5–8).

Class IA phosphoinositide 3-kinase (PI3K) is a heterodimer composed of a p85 regulatory and a p110 catalytic subunit that regulates a variety of cell responses, including cell division and survival. PI3K is activated following Tyr kinase stimulation and by Ras. We found that the C-terminal region of p85, including the C-Src homology 2 (C-SH2) domain and part of the inter-SH2 region, protects the p110 catalytic subunit from Ras-induced activation. Although the p110 activity associated with a C-terminal p85 deletion mutant increased significantly in the presence of an active form of Ras, purified wild type p85-p110 was only slightly stimulated by active Ras. Nonetheless, incubation of purified p85-p110 with Tyr-phosphorylated peptides, which mimic the activated platelet-derived growth factor receptor, restored Ras-induced p85-p110 activation. In conclusion, p85 inhibits p110 activation by Ras; this blockage is released by Tyr kinase stimulation, showing that the classical mechanism of class IA PI3K stimulation mediated by Tyr kinases also regulates Ras-induced PI3K activation.

Experimental Procedures
Cell Culture and Transfections—CTLL2 cells were cultured in RPMI medium containing 10% fetal calf serum and 20 units/ml IL-2. CTLL2 cells were transfected by electroporation using 50 μg of each cDNA as described (36) and collected 24 h later. Stable CTLL2 cell lines (32) were cultured with 2 μg/ml puromycin (Sigma). NIH3T3 cells were cultured...
in DMEM containing 10% calf serum (Invitrogen) and were transiently transfected using LipofectAMINE reagent (Invitrogen) according to the manufacturer’s recommendations. Stably transfected NIH3T3 cells (37) were cultured in DMEM with 10% calf serum and 1 mg/ml G418 (Calbiochem).

cDNAs, Antibodies, and Reagents—The following cDNAs were used for transfections: pEObe-PS5-LoK (38), pSG5 empty vector, pSG5-HAp65C, pCG-HAp95, pSG5-V12Ras, and pSG5-HA-PKB (32). SHP1 cDNA was amplified from poly(A)-mRNA of Jurkat cells and subcloned in pPK5. For in vitro transcription and translation, the following cDNAs were used: pBSSKp50as (38), pSG5-V12-Ras, pSG5p110, pSG5p65, and pSG5p85 (32). Anti-HA (12CA5), -PKB, and -pKB antibodies were purchased from Babco, Santa Cruz Biotechnology, and Novagen, respectively. Anti-human SH3 p85 antibody was from DAKO (CA). Anti-sheep Ras antibody was from Upstate Biotechnology, Inc., and anti-Ras (Ab3) monoclonal antibody was from Oncogene Science. Recombinant IL-2 was from Roche Molecular Biochemicals; PDGF was from Calbiochem.

Immunoprecipitation and Western Blot—Cells were lysed in Triton X-100 lysis buffer (50 mM HEPES containing phosphoinositide (brain extract) micelles (0.5 mg/ml), 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM μg/ml aprotinin, and 10 μg/ml leupeptin). For the association of Ras with PKB, cells were lysed in Brij 96 lysis buffer (20 mM Tris-HE, pH 7.5, 150 mM NaCl, 1% Triton X-100) containing protease and phosphatase inhibitors (1 mM Na3VO4, 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM μg/ml aprotinin, and 10 μg/ml leupeptin). For the association of Ras with PI3K, cells were lysed in Brij 96 lysis buffer (20 mM Tris-HE, pH 7.5, 150 mM NaCl, 1% Triton X-100) containing protease and phosphatase inhibitors (as above). Cell lysates were cleared by centrifugation. Total protein concentration was followed by incubation with 30 μg of total cell extracts were resolved by SDS-PAGE and examined in Western blot using anti-HA. B, control, HA-p85, and HA-p6555RK-expressing CTLL2 cells stimulated with 500 units/ml IL-2 for the times indicated were lysed; 30 μg of total cell extracts were resolved by SDS-PAGE and analyzed in Western blot using anti-phospho-Ser-473 PKB or anti-PKB antibody (indicated). The intensity of the PKB phosphorylation, greater phosphorylation increases leads to transient PKB activation in control and HA-p85-expressing cells, HA-p6555RK-expressing cells showed higher basal PKB phosphorylation, greater phosphorylation increases after IL-2 addition, and slower PKB down-regulation kinetics at later time points (Fig. 1B). Similar results were obtained in NIH3T3 cells expressing p85 or p6555RK and activated with PDGF (Fig. 2). See previous studies (31) indicated that p85 binds Tyr-phosphorylated SHP-1 phosphatase via the C-SH2 domain and that SHP-1 binding mediates down-regulation of both Tyr phosphorylation and PI3K activation. Other authors (40) nonetheless showed a contribution by the N-SH2 domain in p85-SHP-1 association. To examine whether the absence of the C-SH2 domain in p6555RK impaired association to SHP-1, we compared

![Image](https://example.com/image.png)
the ability of p65<sub>PI3K</sub> and p85 to bind to this phosphatase. COS-7 cells were transfected with cDNAs encoding SHP-1 and HA-tagged versions of both regulatory subunits. Transfections were performed in the absence or presence of cDNA encoding a constitutive active version of the Src kinase family member Lck (P505-Lck) (36). The distinct proteins were expressed to a similar extent in the p65<sub>PI3K</sub> and p85 samples (Fig. 3). Moreover, when p65<sub>PI3K</sub> and p85 regulatory subunits were immunopurified with anti-HA Ab, both subunits recruited similar amounts of SHP-1 (Fig. 3). This suggests that p85 binding to SHP-1 is not mediated exclusively by the C-SH2 domain. Minor changes in p65<sub>PI3K</sub> and p85 association to SHP-1 were observed in the absence or presence of P505-Lck, consistent with the moderate effect on SHP-1-p85 complex formation when Jurkat cells were examined prior to and following T cell receptor ligation, which triggers Lck activation (31). Considering that p65<sub>PI3K</sub> binds SHP-1 to a similar extent as p85, the slower PI3K/PKB down-regulation kinetics observed in p65<sub>PI3K</sub>-expressing cells is not related to defective p65<sub>PI3K</sub> association to SHP-1.

We also examined whether the lack of the C-SH2 domain in p65<sub>PI3K</sub> could affect the kinetics of binding and detachment of PI3K to the activated PDGFR (9, 30). The amount of p65<sub>PI3K</sub> bound to the receptor at any time point was lower than that of p85 (not shown), confirming that both SH2 domains contribute to mediating PI3K association to PDGFR (9). In addition, whereas the amount of PDGFR associated to p85 returned to basal levels at 3 h post-stimulation, a small fraction of p65<sub>PI3K</sub> remained PDGFR-associated (not shown). This small difference may not account for the significantly more stable association of PI3K/PKB in cells expressing p65<sub>PI3K</sub>. Despite the open questions, the distinct PI3K/PKB down-regulation kinetics of p85- and p65<sub>PI3K</sub>-expressing cells demonstrates that the p85 C-terminal region contributes to control PI3K signal termination.

**Activated Ras Enhances p65<sub>PI3K</sub>-p110 Activation in Vivo**—p85-p110 class IA PI3K is activated following stimulation of Tyr kinases and by active forms of Ras (1–4, 29). To evaluate which pathway acts more efficiently in p65<sub>PI3K</sub>- compared with p85-expressing cells following GFR stimulation, we deprived cells of GF and examined the consequences of expressing constitutive active forms of Ras or Lck (29, 36). V12-Ras and P505-Lck expression were confirmed by Western blot (not shown). V12-Ras and P505-Lck increased phospho-PKB content in the three CTLL2 lines analyzed (control and p85- and p65<sub>PI3K</sub>-expressing cells; Fig. 4A). V12-Ras nonetheless consistently induced more efficient PI3K/PKB activation in p65<sub>PI3K</sub>-expressing than in control cells and reduced PKB activation in p85-expressing compared with control cells (Fig. 4A). Comparable results were obtained when PKB activation was examined by in vitro kinase assay (not shown). p65<sub>PI3K</sub> and p85 NIH3T3 stable cell lines (32) were also examined. Cells were co-transfected with PKB and either V12-Ras or P505-Lck. P505-Lck mediated PKB activation in both p85- and p65<sub>PI3K</sub>-expressing cells (Fig. 4B). However, V12-Ras induced strong PKB activation in p65<sub>PI3K</sub>-expressing cells and very modest PKB stimulation in p85-expressing cells (Fig. 4B). These observations suggest that the p85a C-terminal region (residues 572-724), absent in p65<sub>PI3K</sub>, impairs Ras-induced p110 activation.

**An N-Terminal Ras Peptide Binds p65<sub>PI3K</sub>-p110 and p85-p110 with Similar Affinity**—To examine the differential effect of V12-Ras on p65<sub>PI3K</sub>-p110 and p85a-p110 complexes, we first analyzed the affinity of these complexes for Ras. Rodriguez-Viciana et al. (29) showed that the p85-p110 complex binds Ras-GTP and that this interaction can be displaced with an H-Ras peptide encompassing residues 17-42. If this peptide displaces H-Ras association to p85-p110, we inferred that this peptide could bind directly p85-p110. We prepared beads covalently linked to the peptide; extracts of p65<sub>PI3K</sub> and p85a-expressing CTLL2 cells were prepared and incubated with the Ras peptide-specific column or a control column. p65<sub>PI3K</sub> and p85 were both retained in the Ras column, with slightly greater retention of p85 than of p65<sub>PI3K</sub> (Fig. 5A). We also examined the interaction of p65<sub>PI3K</sub>-p110 and of p85a-p110 with Ras in vivo. Exponentially growing COS-7 cells expressing HA-p65<sub>PI3K</sub>-p110 and HA-p85a-p110 were lysed in Brij 96-containing buffer. Cell extracts were immunoprecipitated using anti-Ras Ab, and the interaction of Ras with PI3K was examined by Western blot using anti-HA monoclonal Ab. As shown in Fig. 5B, HA-p65<sub>PI3K</sub> and HA-p85 associated with Ras to a similar extent. Formation of this complex was confirmed in COS-7 cells deprived of GF and expressing V12-Ras and either p65<sub>PI3K</sub>-p110 or p85a-p110, which yielded similar results (not shown). These observations confirm that p65<sub>PI3K</sub>-p110 does not show higher affinity for Ras binding than p85-p110.

**The p85 C-SH2 Domain Regulates Ras-induced p110 Activation**—To test whether the p85a C-terminal region (residues 572-724) impairs Ras-induced p110 activation, we compared the action of V12-Ras on p65<sub>PI3K</sub>-p110 and p85-p110 complex activities using purified in vitro translated proteins. p85a-p110 and p65<sub>PI3K</sub>-p110 complexes were translated and translated with similar efficiency (Fig. 6A) and showed similar basal in vitro kinase activity (Fig. 6B), suggesting that p65<sub>PI3K</sub> does not...
activate p110 constitutively. We next evaluated whether incubation with a Tyr-phosphorylated peptide mimicking the activated PDGFR (22) would have a distinct effect on p65PI3K-p110 and p85-p110 complexes. Phosphopeptide addition significantly increased p85-p110 complex activity and had a lower activating effect on the p65PI3K-p110 complex (Fig. 6B). These observations confirmed that a mechanism other than Tyr kinase activation may be responsible for enhanced p110 activation by p65PI3K.

GTP-Ras has been shown to trigger PI3K activation in vitro (29). We next compared the ability of V12-Ras to activate p65PI3K-p110 and p85-p110 in vitro. Both V12-Ras and PI3K molecules were in vitro transcribed and translated (Fig. 6C). V12-Ras moderately enhanced p85-p110 kinase activity but had a significantly higher activating effect on p65PI3K-p110 (Fig. 6D). In different assays, p85-p110 induction by V12-Ras ranged from moderate to none, although p65PI3K-p110 was reproducibly highly responsive to V12-Ras addition. The results show that the p85 C terminus, absent in p65PI3K, protects p110 from V12-Ras-induced activation. To confirm the role of the p85 C-terminal domain in the protection of p110 from Ras action, we examined the consequences of adding V12-Ras on the activity of the p50α-p110 complex. p50α is a natural alternative splicing form of p50α that lacks the N-terminal SH3 and Bcr homology domains but retains the p50α SH2 domains and the inter-SH2 domain (38). Defective V12-Ras-induced p50α-p110 activation (Fig. 6D) confirming that the C-terminal domain impairs Ras action on p110.

**Tyr Phosphorylation Releases p110 from the p85 Inhibitory Action**

We next compared the ability of V12-Ras to activate p65PI3K-p110 and p85-p110 with similar affinity. A, extracts (500 μg) of p65PI3K and p85α-expressing CTLL2 cells were incubated with the control column or a Ras-peptide column. After washing, retained material was recovered by boiling beads in SDS sample buffer, resolved by SDS-PAGE, and analyzed in Western blot using anti-p85 Ab. Total lysates (30 μg) were examined in parallel. B, COS-7 cells were cotransfected with a vector encoding wt-p110 and a vector encoding either HA-p65PI3K or HA-p85. Lysates obtained from exponentially growing cells (30 μg) were resolved by SDS-PAGE and analyzed in Western blot using anti-HA Ab or anti-Ras Ab (indicated). Lysates (250 μg) were also immunoprecipitated (IP) with anti-Ras Ab (indicated), resolved in SDS-PAGE, and gels examined by Western blot using anti-HA antibody. The figure illustrates a representative experiment of three with similar results.

**Effect, Allowing V12-Ras to Activate p110—**The observations reported here suggest that Ras-induced p110 activation is controlled by the C-terminal region of p85. As class IA PI3K activation is selectively induced by receptors that trigger Tyr kinase activation (1–4), it was therefore possible that Tyr kinase stimulation is a prerequisite for V12-Ras-induced p110 activation. To test this hypothesis, we examined whether PDGFR phosphopeptide addition rendered p85-p110 sensitive to V12-Ras-induced activation. Fig. 7A shows the in vitro translated V12-Ras and PI3K complexes. Consistent with the results described above (Fig. 6), V12-Ras alone activated p85-p110 moderately and p65PI3K-p110 more efficiently (Fig. 7B), whereas the PDGFR phosphopeptide alone activated p85-p110 more efficiently than p65PI3K-p110 (Fig. 7B). Nonetheless, the combination of V12-Ras and the Tyr-phosphorylated peptide activated p85-p110 synergistically, whereas activation of p65PI3K-p110 was similar to that observed with V12-Ras alone (Fig. 7B). In conclusion, Tyr-phosphorylated peptide binding to p85 releases the inhibition exerted by the C-terminal region, rendering p110 sensitive to V12-Ras action. We propose that Ras action depends on prior activation of Tyr kinases.

**DISCUSSION**

The observations presented here extend our knowledge of the functional role of the p85 regulatory subunit in the control of PI3K activity, showing that the p85 C-terminal region blocks Ras-induced p110 activation. The data illustrate that Tyr kinase stimulation releases p110 from p85 inhibition, triggering partial p110 activation and sensitizing p110 for further activity increases induced by active forms of Ras. Such a sequential mechanism explains why only receptors that stimulate Tyr kinases trigger class IA PI3K activation (1–4), as active Ras operates only after Tyr kinase induction. This study also clarifies the mechanism by which p65PI3K, a mutation found in human cancer (33–35), contributes to PI3K deregulation and...
p85 regulates Ras-dependent PI3K activation

In contrast to p85-p110, p65\textsuperscript{PI3K}-p110 complexes are susceptible to Ras action in the absence of Tyr kinase activation. In addition, p65\textsuperscript{PI3K}-expressing cells show prolonged PI3K/PKB activation kinetics in vitro. The C-terminal region encompassing residues 572–724, which include the final part of the inter-SH2 domain and the C-SH2 domain, is thus an essential regulatory region of class IA PI3K that enables p85 to control PI3K activation as well as its down-regulation kinetics in vitro.

The existence of an inhibitory activity in the p85 C-terminal region was first postulated in view of the higher PI3K activity detected in p65\textsuperscript{PI3K}-expressing cells (32). Here we characterized the kinetics of this activation in greater detail, and we explored the mechanism responsible for p65\textsuperscript{PI3K}-induced p110 activation. We show that p65\textsuperscript{PI3K} expression affects PI3K activity in the following three ways: increasing basal PI3K activity in quiescent cells, enhancing PI3K activation following GFR stimulation, and delaying p110 down-regulation. Both IL-2R stimulation of CTLL2 cells (Fig. 1) and PDGFR stimulation of NIH3T3 cells (Fig. 2) yielded similar PI3K activation profiles after p65\textsuperscript{PI3K} expression, although the initial PI3K activation peak following GFR ligation was more pronounced in CTLL2 cells. This difference reflects the distinct PI3K recruitment mechanisms used by the two receptors, with IL-2R showing constitutive PI3K association (24, 25) and PDGFR requiring both SH2 domains for efficient PI3K recruitment to the cell membrane (9).

The basal 3-poly-PtdIns content of quiescent p65\textsuperscript{PI3K}-expressing cells is probably related to the fact that a small proportion of p65\textsuperscript{PI3K} is constitutively located at the membrane fraction (32). With regard to the slower PI3K/PKB down-regulation kinetics observed in p65\textsuperscript{PI3K}-expressing cells, we first considered the possibility that p65\textsuperscript{PI3K} could bind the SHP-1 phosphatase to a lesser extent than does p85. Cuevas et al. (31) reported that SHP-1 phosphatase binds p85 via the C-SH2 domain and contributes to the down-regulation of Tyr kinase and PI3K activities. Other authors report (40) that the N-SH2 domain also mediates binding to SHP-1. We found that p65\textsuperscript{PI3K} and p85 bind SHP-1 to a similar extent, supporting the idea that the N-SH2 domain mediates this interaction. In conclusion, a defect in SHP-1 binding does not appear to be the cause of prolonged PI3K activation in p65\textsuperscript{PI3K}-expressing cells. We also considered that 3-poly-PtdIns binds to the C-SH2 domain of p85 with greater affinity than analogues of Tyr-phosphorylated residues (30). It is therefore possible that once PI3K is activated, the 3-poly-PtdIns products bind to the C-SH2 domain of p85, displacing it from the associated Tyr-phosphorylated residues (9). As p65\textsuperscript{PI3K} lacks the C-SH2 domain, this mutant could remain associated to Tyr-phosphorylated GFR for prolonged periods to test this hypothesis, we analyzed the kinetics of p65\textsuperscript{PI3K} and p85 dissociation from the activated PDGFR. While the amount of p85-associated PDGFR returned to basal levels at 3 h post-stimulation, a modest amount of p65\textsuperscript{PI3K} remained PDGFR-associated. This small difference may contribute to prolong PI3K/PKB activation but seems insufficient to account for the significantly more stable activation of PI3K/PKB in cells expressing p65\textsuperscript{PI3K}. In the case of IL-2R stimulation, it is also not obvious how the lack of an SH2 domain would affect PI3K activation kinetics, as p85 binding to the activating Src kinases has not been shown to involve the p85 SH2 domain. Nonetheless, the Src kinases promote formation of an intramolecular complex of the C- and N-SH2 domains, thereby down-regulating PI3K activity. Despite these open questions, the distinct PI3K/PKB down-regulation kinetics of p85- and p65\textsuperscript{PI3K}-expressing cells demonstrates that the p85 C-terminal region contributes to control PI3K signal termination.

The other difference between p85- and p65\textsuperscript{PI3K}-expressing cells is the increased activation intensity of PI3K/PKB following receptor stimulation in the latter cells. To identify the mechanism underlying this enhanced p110 activation, we considered the following two possible routes that normally induce class IA PI3K activation: stimulation of Tyr kinases and acti-
vation of Ras (1–4, 9, 29). Two groups of Tyr kinases activate PI3K, the receptors with intrinsic Tyr kinase activity such as PDGFR (9), and cytosolic Tyr kinases, such as Src family kinase members (27, 41), which are activated in response to cytokine receptor stimulation (42, 43). In the case of PDGFR, PI3K activation requires that the Tyr-phosphorylated receptor bind to the p85 SH2 domains (9). In the case of IL-2R, however, p110 is constitutively bound (24, 25), and the Src kinases associate PI3K through a different mechanism involving its SH3 domain and the p85 Pro-rich regions (41). This interaction between Src kinases and p85 could trigger PI3K activation directly. It has nonetheless also been shown that phosphorylation of p85 Tyr-688 by Abl or Src kinases activates PI3K (27).

By using a constitutive active form of the Src family kinase Lck, we show that this pathway induces PI3K activation in p65PI3K- and p85-expressing cells, despite the lack of Tyr-688 in p65PI3K. This suggests that in addition to Tyr-688 phosphorylation, Src kinases use alternative mechanisms to mediate PI3K activation. Addition of a Tyr-phosphorylated peptide representing the activated PDGFR enhanced both p65PI3K-p110 and p85-p110 activities, although p85-p110 was activated more efficiently. Because neither in vitro phosphopeptide addition nor in vivo P505-Lck expression yielded significantly more active p65PI3K-p110 than p85-p110, we concluded that Tyr kinase activation is not responsible for enhanced p65PI3K-p110 activation in response to GFR stimulation. In contrast, the greater V12-Ras-induced PI3K activation in p65PI3K-expressing cells, as well as the reduced V12-Ras-induced p110 activation in p85-expressing cells (compared with control cells), suggests that p85 interferes with V12-Ras-induced p110 activation. We also examined V12-Ras action on purified in vitro translated p65PI3K-p110 and p85-p110 complexes. V12-Ras addition significantly increased p110 activation in p65PI3K-p110 than in p85-p110 complexes. As class IA PI3K activation is selectively induced by receptors that mediate Tyr kinase activation (1–4), we proposed that Tyr kinase stimulation may be a prerequisite for p85-p110 response to active Ras forms. To test this hypothesis and mimic Tyr kinase pathway activation, we used a peptide representing the region of PDGFR associating with p85, and we show that incubation of p85-p110 with the PDGFR phosphopeptide led to partial p110 stimulation and allowed further p110 activation by V12-Ras. In conclusion, p85 protects p110 from active Ras-induced activation. This protection is affected via the C-terminal domain residues 572–724, which are absent in p65PI3K. Tyr kinase stimulation nonetheless releases p110 from p85 inhibition, yielding p85-p110 susceptible to Ras action.

p110 activation following binding of Tyr-phosphorylated peptides to p85 (or by Tyr phosphorylation of p85) may depend on a conformational change in p85, which could then be transmitted to p110, as elegantly discussed by Dhand et al. (15). Such changes have been reported in p85 in response to phosphopeptide binding (19–21). In addition, Layton et al. (44) show that these peptides induce oligomerization, a mechanism that may also be involved in enzyme activation. The structures of the isolated SH2, SH3, and BH domains have been resolved by crystallography (reviewed in Ref. 45), although only a sequence-based structural prediction has been reported for the inter-SH2 region (15). According to this prediction, the inter-SH2 region folds as an anti-parallel coiled-coil of two α-helices, helix 1 and helix 2 (15). Helix 1 (residues 478–492 of p85α) would mediate primarily the interaction with p110, and helix 2 would stabilize helix 1 structure or orientation. Amino acid sequence comparison showed that the inter-SH2 region has a short basic motif similar to the gelsolin PI(4,5)P2-binding domain (46), which may be part of the lipid-binding pocket of the heterodimeric p85-p110 complex. The conformational change in p85 could unmask the PI(4,5)P2 binding pocket of the PI3K heterodimer. The p65PI3K deletion mutant includes helix 1 and 2 but lacks the terminal inter-SH2 region as well as the remaining p85 C terminus, including the C-SH2 domain (32). This difference is likely to affect the structure and behavior of the inter-SH2 region. According to our observations, Ras action necessarily affects p110 substrate recognition and/or phosphate transfer activity via structural changes in p85. It is therefore possible that p65PI3K-p110 has a structure permissive to Ras action, which is only acquired by p85-p110 complexes after Tyr kinase activation.

The results presented here show how the p65PI3K mutant affects p110 activation, a subject of interest considering that a number of human tumors were recently shown to express p65PI3K-like mutations (33–35). p65PI3K affects p110 activation in several ways. First, p65PI3K yields higher basal PI3K activity. The consequences for cell transformation of increased basal PI3K activity have been demonstrated by examining the phenotype of Pten−/− mice. Pten is a phosphatase that dephosphorylates protein substrates as well as 3-poly-PtdIns. A number of observations nonetheless suggest that the increase in 3-poly-PtdIns in cells with heterozygous Pten loss induces transformation (reviewed in Ref. 47). In support of this view, transgenic mice expressing p65PI3K in T cells show a lymphoproliferative disorder similar to that developed by Pten+/− mice (48, 49). p65PI3K also enhances receptor-induced PI3K activation, facilitating Ras action on p110. Ras activation in p65PI3K-expressing cells would thus yield P13K enzyme-active. p65PI3K also prolongs PI3K activation kinetics. As enhanced PI3K activation facilitates cell cycle entry (50), this action probably contributes to the growth advantage of tumor cells expressing this mutation. However, because p65PI3K action on p110 still requires GFR-derived signals, it does not result in high constitutive PI3K/PKB activation. This property of p65PI3K also confers a cell division advantage, as constitutively high activation of PI3K/PKB interferes with activation of Forkhead transcription factors, required for mitosis progression (50).

In conclusion, we find that the C-terminal region of p85α (residues 572–724) regulates the sequential activation of PI3K by Tyr kinases and by Ras and controls PI3K signal termination. In addition, we present novel mechanisms by which p65PI3K enhances p110 activity, contributing to induction of cell transformation.

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