

# Mechanism of Constitutive Phosphoinositide 3-Kinase Activation by Oncogenic Mutants of the p85 Regulatory Subunit\*

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**p85/p110 phosphoinositide 3-kinases regulate multiple cell functions and are frequently mutated in human cancer. The p85 regulatory subunit stabilizes and inhibits the p110 catalytic subunit. The minimal fragment of p85 capable of regulating p110 is the N-terminal SH2 domain linked to the coiled-coil iSH2 domain (referred to as p85ni). We have previously proposed that the conformationally rigid iSH2 domain tethers p110 to p85, facilitating regulatory interactions between p110 and the p85 nSH2 domain. In an oncogenic mutant of murine p85, truncation at residue 571 leads to constitutively increased phosphoinositide 3-kinase activity, which has been proposed to result from either loss of an inhibitory Ser-608 autophosphorylation site or altered interactions with cellular regulatory factors. We have examined this mutant (referred to as p65) *in vitro* and find that p65 binds but does not inhibit p110, leading to constitutive p110 activity. This activated phenotype is observed with recombinant proteins in the absence of cellular factors. Importantly, this effect is also produced by truncating p85ni at residue 571. Thus, the phenotype is not because of loss of the Ser-608 inhibitory autophosphorylation site, which is not present in p85ni. To determine the structural basis for the phenotype of p65, we used a broadly applicable spin label/NMR approach to define the positioning of the nSH2 domain relative to the iSH2 domain. We found that one face of the nSH2 domain packs against the 581–593 region of the iSH2 domain. The loss of this interaction in the truncated p65 would remove the orienting constraints on the nSH2 domain, leading to a loss of p110 regulation by the iSH2. Based on these findings, we propose a general model for oncogenic mutants of p85 and p110 in which disruption of nSH2-p110 regulatory contacts leads to constitutive p110 activity.**

are classified by their preferred substrates and their mode of regulation (1, 2). Class IA PI3-kinases, which are activated by receptor tyrosine kinases, are heterodimers consisting of an SH2 domain-containing regulatory subunit (p85 $\alpha$ , p85 $\beta$ , p55 $\alpha$ , p50 $\alpha$ , or p55 $\gamma$ ) and a catalytic subunit (p110 $\alpha$ , p110 $\beta$ , or p110 $\Delta$ ).

Class IA PI3-kinases are obligate heterodimers *in vivo*, because the p110 catalytic subunits are labile at 37 °C and are unstable as monomers (3). Dimerization of a p110 catalytic subunit with p85 (or the shorter isoforms) maintains the enzyme in a low activity state, as the regulatory subunit inhibits p110 (3). Subsequent activation of p85/p110 involves a translocation of the normally cytosolic enzyme to a membrane as well as an increase in the specific activity of the dimer. The best understood mechanism for these two events is the high affinity binding of the two SH2 domains in p85 to phosphorylated YXXM motifs in receptor tyrosine kinases or their substrates (4, 5). The activity of p85/p110 dimers is also increased by binding of activated Rac or Cdc42 to the BCR homology domain in the regulatory subunit or by binding of activated Ras to the catalytic subunit (6–8).

Numerous data have implicated PI3-kinases in cancer. Mutations in the 3-phosphoinositide phosphatase PTEN are frequently found in human tumors (9), as is overexpression of the p110 catalytic subunits of p85/p110 (Class IA) PI3-kinases (10). These overexpressed p110 subunits are presumably active by virtue of excess free p85 that exists in many cells types (11). Mutational hotspots in p110 $\alpha$  have also been described in human colon cancer (12), and these mutants show increased PI3-kinase activity *in vitro* (13). In addition, a deletion mutant of the p85 regulatory subunit has been discovered in human ovarian and colon cancer (14). This deletion maps to a region of p85 adjacent to an inhibitory serine autophosphorylation site (Ser-608) (15), and it has been suggested that loss of autophosphorylation at this inhibitory site might contribute to oncogenesis (16). Similarly, Jimenez *et al.* (17) described a transforming mutant of murine p85 in which residues 572–724 of p85 are replaced by a non-catalytic fragment from the Eph tyrosine kinase. This mutation leads to constitutive activation of PI3-kinase. A truncated p85-(1–571) mutant (referred to as p65) also leads to constitutive increases in PI3-kinase activity in transfected cells, suggesting that the fragment from Eph does not contribute to activation. The phenotype of the p65 mutant has led to a proposal that the deleted residues 572–724, which contain the end of iSH2 and the C-terminal SH2 domain, comprise an inhibitory domain whose removal leads to a loss of interactions with cellular inhibitors (18). Alternatively, it has

Phosphoinositide 3 (PI3)<sup>1</sup>-kinases are critical regulators of cell growth, motility, and survival. Phosphoinositide 3-kinases

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<sup>1</sup> The abbreviations used are: PI3-kinase, phosphoinositide 3-kinase;

GST, glutathione S-transferase; nSH2, N-terminal SH2 domain; iSH2, inter-SH2 domain.

been suggested that the constitutively activated phenotype of the truncated p65 is due to the fact that p65/p110 dimers can be activated by GTP-Ras in the absence of SH2 domain occupancy, whereas Ras activation of wild-type p85/p110 requires SH2 domain occupancy (19).

An alternative explanation for the increased PI3-kinase activity caused by the p65 oncogenic mutant would be that truncation at residue 572 disrupts the native positioning of the nSH2 domain so that it can no longer regulate p110 activity. We have previously shown that the minimal regulatory fragment of p85 is the N-terminal SH2 domain (nSH2) linked to the inter-SH2 domain (iSH2) (20). The iSH2 domain is a rigid 100 Å antiparallel coiled-coil (21, 22) that mediates binding between p85 and the N terminus of p110 (21, 23–25). However, iSH2 binding has no effect on p110 activity. In contrast, the nSH2-iSH2 fragment (hereafter referred to as p85ni) inhibits p110 and mediates phosphopeptide-induced activation of the p85ni/p110 dimer (20). Importantly, phosphopeptide binding to the SH2 domain of p85ni has no effect on the conformation of the iSH2 domain (21, 22, 26). Given (a) the conformational rigidity of the iSH2 domain and (b) the fact the p110 is inhibited by p85ni but not by the iSH2 domain alone, we have proposed a model in which the iSH2 domain serves as a rigid tether linking p85 and p110 and facilitates a second, regulatory interaction between the nSH2 and p110. Phosphopeptide activation of p85/p110 would involve a change in or disruption of this inhibitory nSH2-p110 contact.

To test whether the activating effects of the p65 truncation could be due to intrinsic changes in the regulation of p110 activity by the nSH2 domain rather than loss of inhibitory inputs from Ser-608 or cellular factors, we have examined the effects of wild-type *versus* truncated p85 and p85ni on p110 activity. Interestingly, we found that truncated mutants of both p85 and p85ni bind p110 but do not inhibit p110. These data provide a simple explanation for the activated phenotype of p65, in which the truncated molecule stabilizes p110 in its high activity state. To understand, at a structural level, how truncation of p85 could affect nSH2-mediated inhibition of p110, we determined the binding interface between the nSH2 and iSH2. In an approach that should have general application in the structurally challenging task of measuring interdomain positions in multidomain proteins and complexes, site-specific spin labeling was combined with heteronuclear NMR spectroscopy to measure a network of distances between the nSH2 domain and the region of the iSH2 domain affected by the p65 truncation. We found that residues 581–593 of the iSH2 domain were in close contact with the nSH2 domain, which folds down over this region of the iSH2 domain. Both the functional and the structural results suggest that the distal end of the iSH2 domain is critical for maintaining the nSH2 domain in a defined, inhibitory conformation relative to p110.

#### EXPERIMENTAL PROCEDURES

**Mutagenesis and Preparation of Recombinant Proteins and Analysis of p85-p110 Binding**—Human p85 mutants were produced by standard 4-primer PCR techniques. All mutants were confirmed by sequencing. Human p85 $\alpha$  or fragments were expressed as GST fusions as previously described. GST-p85 $\alpha$  and GST-p65 (residues 1–571) were used directly, whereas GST was removed from p85ni (residues 320–600) and p85ni(572stop) (residues 320–571) by cleavage with thrombin and absorption on glutathione-Sepharose (Amersham Biosciences). All proteins were >95% pure by SDS-PAGE and Coomassie Blue staining. All fragments were tested for p110 $\alpha$  binding and inhibition as previously described (22). In the binding studies, tracer amounts of Myc-p110 $\alpha$  were incubated with excess immobilized GST-p85 or mutants thereof, washed, and assayed by anti-Myc blotting. Recombinant Myc-tagged p110 $\alpha$  was produced in baculovirus-infected Sf-9 cells or transfected human embryonic kidney 293T cells as previously described (3). Phosphopeptide activation of p85/p110 dimers by a YXXM phosphopeptide

derived from the platelet-derived growth factor receptor was as previously described (20).

**Isotopic and Nitroxide Spin Labeling of Recombinant p85 Fragments**—Uniformly  $^{15}\text{N}$ - or  $^2\text{H}^{13}\text{C}^{15}\text{N}$ -labeled p85ni was bacterially expressed in minimal medium supplemented with either  $^{15}\text{NH}_4\text{Cl}$  alone or  $^2\text{H}_2\text{O}$ ,  $^2\text{H}^{13}\text{C}$ -glucose, and  $^{15}\text{NH}_4\text{Cl}$  for isotopic incorporation. Purified  $^{15}\text{N}$ -labeled p85 fragments containing single cysteine residues at various positions were labeled with 3-maleimido-proxyl (Aldrich) dissolved in  $\text{Me}_2\text{SO}$  at a molar ratio of 1:20 and a final  $\text{Me}_2\text{SO}$  concentration of 0.5%. The samples were rotated at room temperature for 5 h, dialyzed extensively against phosphate-buffered saline, pH 7.4, and concentrated in Centrprep concentrators (Amicon, Bedford, MA).

**NMR Spectroscopy and Structure Calculation**—The NMR samples contained 0.5–2.0 mM p85ni in phosphate-buffered saline with 0.5% Triton X-100 at pH 7.4. All NMR data were processed and analyzed using NMRPipe (27) and NMRView (28). The nSH2 backbone resonances were assigned using three-dimensional TROSY-based HNCN/HN(CA)CO and HNCACB/HN(CO)CACB data sets (29) from a  $^2\text{H}^{13}\text{C}^{15}\text{N}$ -labeled p85ni sample collected at 27 °C on a Bruker 800 MHz spectrometer at the New York Structure Center. The interdomain paramagnetic relaxation effects in spin-labeled, uniformly  $^{15}\text{N}$ -labeled p85ni samples were analyzed using 600 MHz  $^1\text{H}^{15}\text{N}$  TROSY spectra (30) of both the oxidized and 3 mM phenylhydrazine-reduced samples. The intensities of all assigned nSH2 resonances were measured in the reduced and oxidized samples and used with the  $\tau_c$  value of 17 ns calculated from  $\{^1\text{H}\}^{15}\text{N}$  relaxation data to define broad distance constraint ranges of (i) <14 Å, (ii) >20 Å, or (iii) ambiguous and therefore not included as a constraint, as described (31).

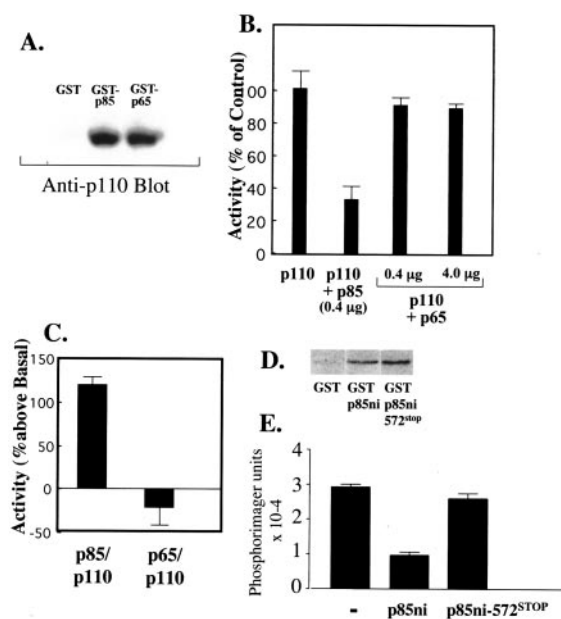
For the structure calculations using XPLOR-NIH (32), the known structures of the nSH2 (33) and a recent crystal structure of the iSH2<sup>2</sup> were treated as rigid bodies connected by the unconstrained linking residues 424–434; varying the length of the unconstrained segment by 5 residues in either direction had no effect on the calculated structures. A random set of 100 starting orientations was generated by high temperature torsion angle dynamics equilibration at 7,500 K. A rigid body simulated annealing protocol, in which the distance constraints were phased in over the course of a second equilibration at 5,000 K and maintained during cooling to 50 K, was then used to calculate a family of structural models that satisfied all distance constraints to within 0.5 Å from all 100 starting structures. The rigid body constraints were then removed, and the final structures were energy minimized to optimize side chain packing. Only those relaxed structures that still satisfied the spin-label distance constraints were retained for analysis.

#### RESULTS AND DISCUSSION

**Truncated p85-(1–571) Binds but Does Not Inhibit p110**—To directly examine the effect of the oncogenic truncation of p85 (17) on the activity of p110, we produced recombinant GST-p85 in both wild-type and truncated p65 forms and tested their ability to regulate p110. As previously shown, full-length GST-p85 bound and inhibited p110 (Fig. 1, A and B), and the activity of GST-p85-p110 dimers was increased by a phosphotyrosine peptide derived from the platelet-derived growth factor receptor (Fig. 1C). In contrast, p65 bound to p110 but had no effect on its activity, and the activity of p65/p110 dimers was not altered by phosphopeptide (Fig. 1, A–C). Thus, p65 binds but does not inhibit p110, providing a simple explanation for its activated phenotype when expressed in cells.

The constitutively active phenotype of p65 (17) and a  $\Delta$ 582–605 mutant of human p85 (14) have been attributed to a loss of inhibitory input from the distal iSH2 domain and the cSH2 domain (18) or the loss or disruption of the inhibitory Ser-608 phosphorylation site (16). To test these possibilities, we compared the regulation of p110 by p85ni *versus* p85ni(572stop), which terminates at the p65 truncation site. Importantly, we find that p85ni inhibits p110, whereas the truncated p85ni(572stop) binds p110 but has no effect on p110 activity (Fig. 1, D and E). These data show that the 572 truncation leads to a loss of p110 inhibition within the context of either intact p85 or p85ni. Thus, the effect of the truncation cannot be

<sup>2</sup> N. Miled, Y. Yang, O. Perisic, M. Zvelebil, J. M. Backer, and R. Williams, submitted for publication.



**FIG. 1. Oncogenic mutants of p85 bind, but do not inhibit, p110.** *A*, GST, GST-p85, or GST-p65 were immobilized on glutathione-Sepharose beads and incubated with recombinant Myc-tagged p110 $\alpha$  produced in baculovirus-infected Sf-9 cells. The beads were washed and blotted with anti-Myc antibodies. *B*, recombinant p110 $\alpha$  was incubated in the absence or presence of p85 or p65 (0.4 or 4  $\mu$ g) for 1 h at 4  $^{\circ}$ C and assayed for lipid kinase activity. *C*, p85/p110 and p65/p110 dimers were incubated in the absence or presence of 100  $\mu$ M YXXM phosphopeptide for 30 min at 4  $^{\circ}$ C and assayed for lipid kinase activity. The data are plotted as -fold stimulation. *D*, GST, GST-p85ni, or GST-p85ni(572stop) were immobilized on glutathione-Sepharose beads and incubated with recombinant Myc-tagged p110 $\alpha$ . The beads were washed and blotted with anti-Myc antibodies. *E*, recombinant p110 was incubated with p85ni or p85ni(572stop) for 1 h at 4  $^{\circ}$ C and assayed for lipid kinase activity.

because of the loss of the cSH2 domain or the Ser-608 phosphorylation site, as these are not present in p85ni. Instead, these data suggest that the loss of residues 571–600 in the iSH2 domain might lead to a disruption of the native nSH2 domain orientation, such that it no longer forms an inhibitory contact with p110.

**Mapping the Interactions between the nSH2 and iSH2 Domains**—Although inhibition of p110 by p85ni requires residues 572–600 (Fig. 1E), these residues are not sufficient to inhibit p110, as we have previously shown that an iSH2-cSH2 construct binds but does not inhibit p110 (34). Thus, the inhibitory function of residues 572–600 only occurs in constructs containing the nSH2 domain. We therefore decided to map contacts between the nSH2 domain and the proximal and distal ends of the iSH2 domain by measuring the paramagnetic relaxation effects from site-specific spin labels in the iSH2 domain on assigned amide proton NMR resonances in the nSH2 domain.

Backbone amide proton and nitrogen resonances of the nSH2 domain were assigned from triple resonance three-dimensional TROSY spectra of perdeuterated,  $^{13}\text{C}^{15}\text{N}$ -labeled wild-type p85ni. Based on our modeling and EPR studies (22) and a recent crystal structure of the iSH2 domain,<sup>2</sup> we produced mutants of p85ni containing single cysteine residues at residues 448 and 449 near the N-linker region that joins the nSH2 and iSH2 domains and at residues 575, 582, 584, 591, and 596 in the region whose deletion causes a loss of p110 inhibition (Fig. 1). None of the point mutations affected binding or inhibition of p110 (data not shown).  $^{15}\text{N}$ -labeled samples of each single cysteine mutant were labeled with 3-maleimido-PROXYL. For each mutant,  $^1\text{H}^{15}\text{N}$  TROSY spectra were acquired for both the paramagnetic sample and a control in which

the nitroxide spin label was chemically reduced to its diamagnetic form. Paramagnetic relaxation from an introduced nitroxide spin label leads to distance-dependent broadening (Fig. 2, top panel) and hence a conveniently measured reduction in cross-peak intensity for amide protons within 20  $\text{\AA}$  of the nitroxide radical (31). Of the spin-labeled sites, the most extensive relaxation effects were observed for position 584, and moderate effects were observed for positions 448, 582, and 591 (Fig. 2, lower panel). Few effects were seen for positions 449, 575, and 596 (data not shown). Using the naming convention of Nolte *et al.* (35), the relaxation effects from the spin labels were concentrated on one face of the nSH2 domain, including the N and C termini, the AA and AB loops, strand D/D', the EF loop, and the far end of the BG loop through strand G (at some distance from the region of the BG loop involved in phosphopeptide binding (35)). These data define a face of the nSH2 domain that packs against the C-terminal helices of the iSH2 domain near residue 584.

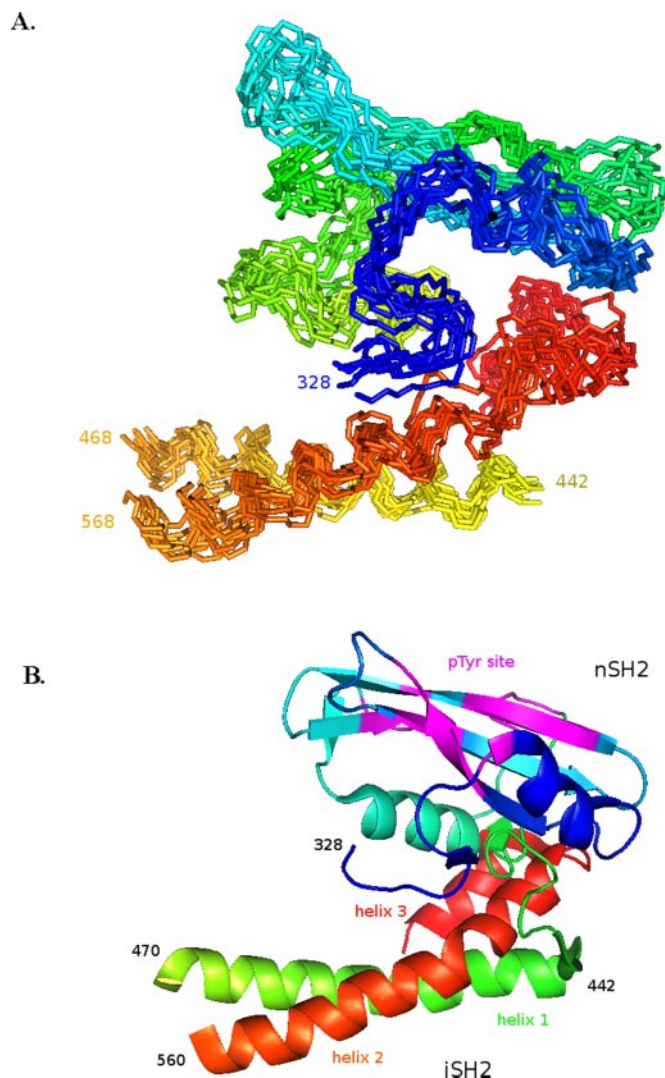
To visualize the interactions between the nSH2 and iSH2 domains, the paramagnetic relaxation effects were used to position the known structure of the nSH2 domain (35–38) with respect to the newly solved structure of the iSH2 domain.<sup>2</sup> The paramagnetic relaxation effects were converted into broad distance restraint ranges, and the nSH2 and iSH2 domains were treated as rigid bodies. A typical simulated annealing protocol was used to derive structural models consistent with the restraints. A single family of models with root mean square deviations for the backbone atoms of 1.6  $\text{\AA}$  (Fig. 3A) was found that satisfied all restraints. On viewing a representative structural model (Fig. 3B) it is apparent that the nSH2 domain only interacts with the C-terminal helices of the iSH2 coiled-coil. Residues 329–333 and 403–410 of the nSH2 domain pack against the exposed face of the C-terminal iSH2 helix-turn-helix extending from residue 581–593; the phosphopeptide-binding site of the nSH2 domain is oriented away from the nSH2-iSH2 contact. The interdomain contact observed here is likely to be important in maintaining the relative orientations of the nSH2 and iSH2 domains.

The p85 and p55 subunits of Class IA PI3-kinases are inhibitors of the p110 subunit, maintaining it in a low activity state (3). Many protein kinases are regulated by association with inhibitory subunits. In protein kinase A, activation involves the cAMP-stimulated dissociation of the inhibitory regulatory subunit from the catalytic subunit (39). For Class IA PI3-kinases, however, the two subunits cannot dissociate, as p110 monomers are unstable (3). Thus, regulation of p110 is caused by an allosteric mechanism involving the direct binding of an activator to p110 (in the case of GTP-Ras) (40) or to p85 (in the case of receptor tyrosine kinases or their substrates) (5). In the latter case, conformational changes initiated by the occupancy of the p85 SH2 domains are transmitted to p110 through alterations in inhibitory contacts at the p110-p85 interface.

We have recently proposed a model of p85 regulation of p110 in which the primary contact between p85 and p110 is a conformationally rigid interface involving the N terminus of p110 and helices 1 and 2 of the iSH2 domain (34). This primary binding site facilitates a secondary interaction between the nSH2 and p110 that is required for inhibition of p110 (22). The inhibitory interface is presumably modified or abolished when phosphopeptides occupy the nSH2 domain. This model is supported by the fact that the nSH2 domain is specifically required for inhibition of p110 (20, 34) and that we and others have been unable to detect significant conformational change within the iSH2 domain upon phosphopeptide binding to the SH2 domains (22, 26, 34).

To probe for structural interactions between residues 571–

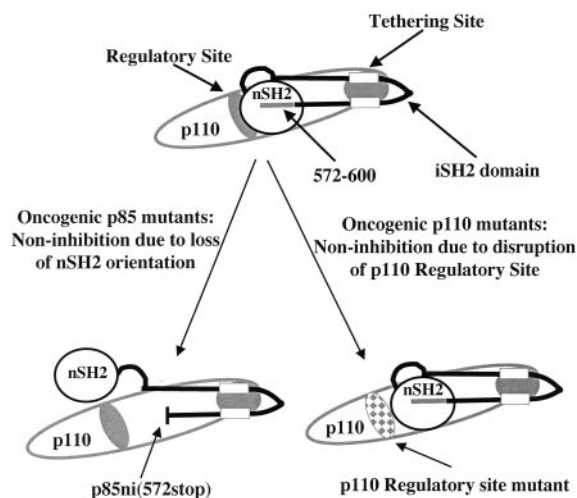




**FIG. 3. Orientation of nSH2 domain with respect to the iSH2 domain.** *A*, side view of the family of structural models for p85ni that best fit the spin label distance constraints between nSH2 and iSH2, colored from *blue* at the N terminus to *red* at the C terminus. *B*, single conformer of the p85ni model, colored as above, but with residues that contact phosphopeptide shown in *magenta*. All views were produced using Pymol (48).

cation of an interface between one face of the nSH2 domain and residues 581–593 of the iSH2 domain. These contacts are likely to be important for establishing the relative orientations of the two domains and for maintaining the nSH2 domain in its inhibitory orientation relative to p110.

It is interesting to consider mutations of p85 found in human and murine cancer in the light of our structural data. These mutants include the p65 truncation (17) and the replacement of residues 582–605 with a single Ile, found in human colon and ovarian cancer (14). Previous studies have suggested that the activated phenotype of these mutations is due to altered interactions with cellular regulatory proteins (18, 19). However, we have clearly shown that the effects of the p65 truncation can be detected *in vitro* with recombinant proteins. Alternatively, it has been proposed that the activating phenotype of p85 mutations could be due to a loss of inhibition from the Ser-608 autophosphorylation site (14, 16). However, we have demonstrated that the activating effect of the p65 truncation is still observed within the context of the p85ni, which does not contain Ser-608. Thus, although Ser-608 may be an important regulatory site for p85/p110 *in vivo*, removal of the 572–600



**FIG. 4. Model of PI3-kinase activation by oncogenic mutations.** p85 and p110 dimers are held together by a conformationally rigid interface involving the N terminus of p110 and the iSH2 domain of p85. A distinct regulatory interface involves the nSH2 domain of p85 and a second region of p110. Mutations in p85 that cause a loss of p85 orientation (e.g. in the p65 truncation) or mutations in p110 that cause a disruption of the nSH2-p110 interface (e.g. in helical domain mutants of p110) lead to a loss of p110 inhibition and constitutive PI3-kinase activity.

region of p85 leads to a constitutively active (*i.e.* non-inhibited) p110 *in vitro*, independently of effects from Ser-608.

In contrast to these previous studies, our work provides a structural explanation for the phenotype of oncogenic p85 mutants. The truncation of p85 or p85ni at residue 571 would eliminate residues 581–593, the region of close nSH2-iSH2 contacts. The 582–605 deletions described in human cancer (14) would be predicted to have a similar effect. Our structural modeling suggests that these mutations would significantly disrupt nSH2-iSH2 interactions, causing a shift of the nSH2 domain away from its normal orientation. In both cases, it is likely that the loss of nSH2-iSH2 contacts would disrupt regulatory contacts between the nSH2 domain and p110, leading to a constitutively active phenotype.

Although the sites of nSH2-p110 contact have not yet been defined, a mutational hotspot has been identified in the helical domain of human p110 $\alpha$  in human colon cancer (12). A recent crystal structure of the iSH2 domain complexed with the N terminus of p110, combined with the previously solved structure of the p110 $\gamma$  catalytic domain (47), has allowed the modeling of the intact p85-p110 dimer.<sup>2</sup> In this model, the nSH2 domain is in contact with the helical domain of p110. Consistent with this model, we have found that “hot spot” helical domain mutants of p110 (E542K, Q546K, E545K) bind to p85ni, but are not inhibited by p85ni.<sup>2</sup> These data suggest that helical domain p110 mutations lead to a disruption of a regulatory interface with the nSH2 domain of p85. Such mutations would be mechanistically analogous to the effect of the p65 truncation, which also disrupts nSH2-mediated regulation of p110 (Fig. 4). Thus, we propose that oncogenic mutations of both p85 and p110 may share a similar mechanism of action, loss of inhibition of p110 by p85.

Although our data are consistent with this model of p110 regulation, we have not directly demonstrated that removal of residues 572–600 leads to a change in nSH2-p110 interactions. It is possible that both the nSH2 domain and residues 572–600 form independent inhibitory contacts with p110, neither of which is sufficient for inhibition. In this alternative model, given the close contacts between the nSH2 domain and residues 583–592, phosphopeptide binding to the nSH2 domain

could lead to conformational changes affecting the interactions of both regions with p110. Footprinting studies to definitively map the sites of nSH2-p110 contact and their modulation by phosphopeptides are in progress.

Carrera and co-workers (19) have shown that whereas phosphopeptide occupancy of p85 SH2 domains is a prerequisite for activation of p85/p110 dimers by Ras-GTP, activation of p65/p110 dimers by Ras can occur in the absence of phosphopeptide. Our data are consistent with this finding, which suggests that truncation of p85 at residue 572 mimics activation by phosphopeptide. This is because in p65 the absence of inhibitory nSH2-p110 contacts mimics the loss/alteration of inhibitory contacts that occurs upon phosphopeptide binding to p85/p110. The finding that phosphopeptide binding is not required for Ras activation of p65 does not mean that residues 572–600 are involved in interactions with Ras but rather that Ras preferentially interacts with disinhibited (or in the case of p65, non-inhibited) p110. These data increase the physiological importance of the nSH2-mediated regulation of p110, because they suggest that phosphopeptide-mediated disinhibition is a prerequisite for additional activation by Ras.

In summary, inhibition of p110 specifically requires the nSH2 domain. Using a combined spin label/NMR methodology, we have shown that inhibition of p110 requires that the nSH2 domain be properly positioned by contacts with the 581–593 region of the iSH2 domain. Deletion of this region in oncogenic mutants of p85 leads to a loss of p110 inhibition. Our data provide a simple mechanism for the activating effects of oncogenic p85 mutants and suggest a general model for oncogenic mutants of p85 and p110.

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