Structural comparisons of class I phosphoinositide 3-kinases

L. Mario Amzel, Chuan-Hsiang Huang, Diana Mandelker, Christoph Lengauer, Sandra B. Gabelli and Bert Vogelstein

Abstract | Class I phosphoinositide 3-kinases (PI3Ks) are lipid kinases that regulate cell growth. One of these kinases, PI3Kα, is frequently mutated in diverse tumour types. The recently determined structure of PI3Kα reveals features that distinguish this enzyme from related lipid kinases. In addition, wild-type PI3Kγ differs from PI3Kα by a substitution identical to a PI3Kα oncogenic mutant (His1047Arg) that might explain the differences in the enzymatic activities of the normal and mutant PI3Kα. Comparison of the PI3K structures also identified structural features that could potentially be exploited for the design of isoform-specific inhibitors.

Phosphatidylinositol 3,4,5-trisphosphate (PIP_3) is a minor component of cellular membranes that can initiate signalling events that control the growth of normal as well as neoplastic cells. For example, PIP_3 acts as a docking site for pleckstrin homology domain-containing proteins, such as the Akt (also known as protein kinase B (PKB)) serine/threonine kinases, and for the 3-phosphoinositide-dependent protein kinase 1 (PDK1)[1]. Once associated with the membrane, AKTs are activated by phosphorylation at two sites and in turn phosphorylate numerous protein targets, including mTOR (also known as FRAP1), tuberin (TSC2), glycogen synthase kinase 3 β (GSK3β), BAD, MDM2, p21(encoded by *CDKN1A*), caspase 9, inhibitor of NFκB kinase (IKK, also known as CHUK), and a subset of forkhead transcription factors (for reviews see REFS 2–4). The biological consequences of Akt activation are broad, and include regulation of cell proliferation, survival and motility. The class I PI3Ks (PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ) are lipid kinases that phosphorylate phosphatidylinositol 4,5-bisphosphate (PI 4,5-P_2) at the 3 position of the inositol ring, generating PIP_3 (REFS 1,4–6).

In addition to their importance in general signal transduction, PI3Ks have an important role in disease, particularly in cancers[7–10]. Recently, it has been shown that *PIK3CA* (which encodes p110α, the catalytic subunit of PI3Kα) is somatically mutated in diverse cancers, including those of the colon, rectum, breast, brain, liver and ovary[11–19]. Deletion and truncation mutations in the regulatory subunit p85α of PI3K have also been found in human tumours, although less frequently than those in the catalytic subunit[20].

Because mutations in the catalytic domain (p110α) of PI3Kα that constitutively activate its kinase activity are common in cancers, many groups have targeted this enzyme for drug development[21]. Most of the compounds characterized, however, are not specific and inhibit other PI3Ks as well as other kinases. Availability of the structure of the various PI3K enzymes could facilitate the development of more specific inhibitors. The structures of wild boar and human p110γ (PI3Kγ) are known[22–24], as is the structure of a complex between the amino-terminal domain of p110α (residues 1–108) and the human p85α inter (i)SH2 domain (residues 431–600)[25]. The structure of a complex between the full-length human p110α catalytic subunit and the domains of the p85α regulatory subunit that is crucial for its binding has recently been reported[26]. The PI3Kα and PI3Kγ structures, coupled with information about the sequence and biochemistry of the PI3K enzymes, provide a wealth of information about conserved features as well as distinctive characteristics between members of this important family of enzymes.

The class I PI3K gene family

Two subclasses of class I PI3K enzymes have been described. Class IA enzymes are composed of catalytic subunits whose enzymatic activities are completely dependent on their binding to regulatory subunits. Human cells contain three genes (*PIK3CA*, *PIK3CB* and *PIK3CD*) that encode the catalytic subunits of class IA PI3K enzymes (termed PI3Kα, PI3Kβ and PI3Kδ, respectively). The major polypeptides produced by these three genes are p110α, p110β and p110δ, respectively, collectively termed p110. p110α and p110β are expressed in most tissues, whereas p110δ is expressed primarily in leukocytes and in a small number of other cell types. The regulatory subunits of class IA enzymes are collectively referred to as p85 and are encoded by three genes in humans (*PIK3R1*, *PIK3R2* and *PIK3R3*). *PIK3R1* encodes p85α, p55α and p50α as a result of alternative splicing, whereas *PIK3R2* encodes only p85β and *PIK3R3* encodes only p55γ. The p85α and p85β polypeptides are expressed in most cells, whereas the other isoforms are expressed in a more limited manner.

The p110 subunits of class IA PI3Ks have five domains: an N-terminal domain called ABD (adaptor binding domain) that binds to the regulatory p85 family members, a Ras binding domain (RBD), a C2 domain that has been proposed to bind to cellular membranes, a helical domain of unknown function and a kinase catalytic domain. The p85 polypeptides also have five known domains: an N-terminal SH3 domain, a Rho GTPase-activating protein (GAP) domain, and two SH2 domains (the more N-terminal nSH2 and the C-terminal cSH2) separated by an iSH2 domain that is responsible for binding to the catalytic subunit. In the basal state, the p85 regulatory subunits bind to and inhibit the p110 catalytic subunits. Following appropriate cellular stimuli, the nSH2 and cSH2 domains bind phosphorylated tyrosines in activated receptors and adaptor proteins, and this phosphotyrosine binding activates the p110 catalytic subunits. It is important to note that the phosphotyrosine binding does not release p85 from p110: the heterodimeric state persists after phosphopeptide binding.

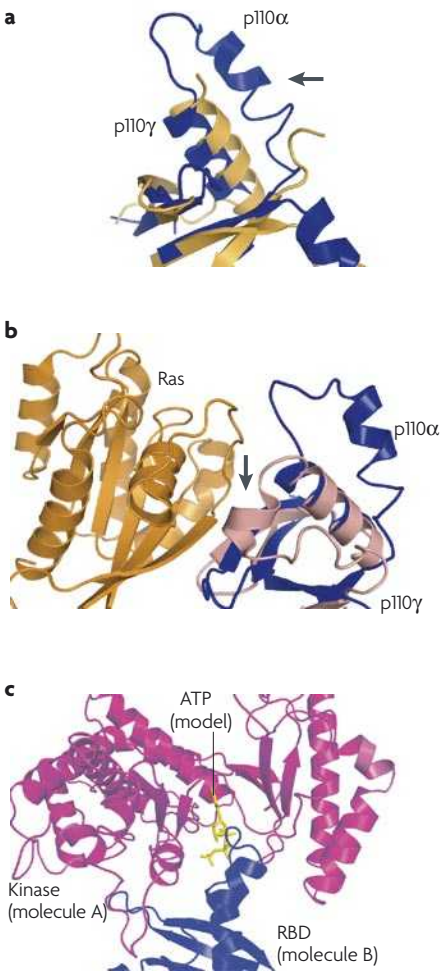

Figure 1 | **Comparison of the Ras-binding domains (RBDs) of p110α and p110γ.** **a** | Comparison of the RBDs of p110α (blue) and free p110γ (gold). The arrow shows the ordering of residues 227–247 in p110α. **b** | Comparison of the RBDs of p110α and Ras–p110γ complex. The arrow indicates the position of residues 255–267 in p110γ that become ordered by the binding of Ras. **c** | Interaction between p110α molecules in the crystal structure. The RBD (blue, molecule B) is locked in the ATP binding pocket of the kinase domain from a neighbouring molecule (magenta, molecule A).

The class IB PI3K consists of only one enzyme, PI3Kγ. It does not contain the same N-terminal p85-binding motif and does not need to interact with a regulatory subunit in order to be enzymatically active. Instead, it appears to be activated by G-protein-coupled receptors and regulated by hetero-trimeric G proteins. The catalytic subunit of PI3Kγ, p110γ, is encoded by *PIK3CG* and is expressed in leukocytes and in a small number of other tissues. The p110γ polypeptide interacts with adaptor subunits called p101 and p84/87 (REFS 27–29), which might help tether p110γ to the membrane and facilitate its interaction with G proteins. The p110γ polypeptide can also bind Ras in a fashion similar to the class IA catalytic subunits.

Structure of class I PI3Ks

Mutation sites in the ABD of p110α make different contacts than other class IA PI3Ks. Although the sequences of p110β and p110δ are 55% identical, they are only 40% identical to p110α. In p110α–niSH2 (REF. 26), the ABD and kinase domains interact with each other through close contacts between arginine (Arg) 38 and Arg88 from the ABD and glutamine (Gln) 738, aspartic acid (Asp) 743 and Asp746 from the kinase domain[26]. Based on this observation, the mutations at Arg38 and Arg88 that are found in cancers were proposed to alter the catalytic activity of PI3Kα by affecting this ABD–kinase domain interaction[26]. Is this ABD–kinase domain interaction a common feature of all class IA PI3Ks? Surprisingly, although Arg38 and Arg88 are conserved in all the ABDs of class IA PI3Ks, the residues of the kinase domain with which they interact are not: Gln738, Asp743 and Asp746 of p110α are replaced by cysteine, alanine and glutamic acid (Glu) in p110β and p110δ (see Supplementary information S1 (figure)). These replacements highlight a crucial difference in the intramolecular regulatory motifs in PI3Ks that distinguishes PI3Kα, the isoform that is frequently mutated in cancers, from the other class IA PI3Ks. It explains why mutations at these positions are more frequent in PI3Kα than in the other isoforms: analogous mutations in the other PI3K isoforms would probably have no effect on the kinase domain.

Comparisons among structures

RBD. Residues 255–267 within the RBD domain of p110γ are not ordered in the structure of the free enzyme but are ordered in the structure of the complex between p110γ and Ras[22]. On the basis of this observation it was proposed that Ras binding results in ordering of this mobile loop[22]. However, the corresponding region (residues 227–247) of p110α is ordered in the absence of Ras and is in a conformation different from that in the p110γ–Ras structure (FIG. 1a, 1b). In fact, part of this loop is locked in the ATP binding site of the kinase domain of a neighbouring molecule in the p110α–niSH2 crystal (FIG. 1c)[26]. This interaction might be a crystallization artefact but could represent a true physiological interaction. We hypothesize that *in vivo* such an interaction could lead to intermolecular inhibition of p110α: the RBD loop of one molecule competes with ATP binding to another molecule by occupying the binding site. Ras could then activate p110α by releasing the RBD loop from the ATP binding site, providing a mechanism of Ras activation that is different from the one operational in p110γ. Arguing against this hypothesis is the fact that gel filtration chromatography of the purified p110α–niSH2 complex carried out at a protein concentration of 0.5 mg/ml shows a single symmetrical peak corresponding to a molecular weight of 150 kDa, consistent with a soluble monomer (C.H.H., S.B.G. and L.M.A., unpublished observation). This might indicate that this is a low-affinity interaction that becomes relevant only when PI3Kα is associated with the membrane.

If the interaction between the RBD and the kinase domain that is observed in the crystal is physiological, how is the formation of the infinite chain of molecules found in the crystal prevented? A possible solution to this conundrum is provided by the observation that parts of p85α, including cSH2, are omitted in our construct. Were these parts present, they could clash with a third molecule, allowing only two molecules to interact with each other and precluding concatemerization.

Based on the comparison of the structures of free p110γ and the Ras–p110γ complex, Pacold *et al.* identified an allosteric effect that is induced by the binding of Ras to p110γ (REF. 22). In particular, they suggested that the C2 and kinase domains 'spread apart' in the Ras-bound structure. In the p110α–niSH2 structure, the C2 domain appears to be even farther apart from the kinase domain than in the Ras-bound p110γ, suggesting that p85 binding to p110α has an effect similar to, or even greater than, that induced by the Ras binding to p110γ, but in p110α this effect does not enhance the catalytic activity[30].

C2 domain. The interaction of p110α with its regulatory subunit p85α involves close contacts between the C2 domain of p110α and the iSH2 domain of p85α. One of the C2 domain residues responsible for this interaction, asparagine (Asn) 345, which is mutated to lysine (Lys) in some cancers, is located on a loop (calcium binding region 1 (CBR1), residues 342–355). There is no sequence similarity in the CBR1 loops of p110α and p110γ, even though the region preceding the loop contains the conserved sequence Arg–(isoleucine (Ile)/valine (Val))–Lys–Ile (FIG. 2). It is not known whether the C2 domain of the class IB p110γ also contacts its regulatory

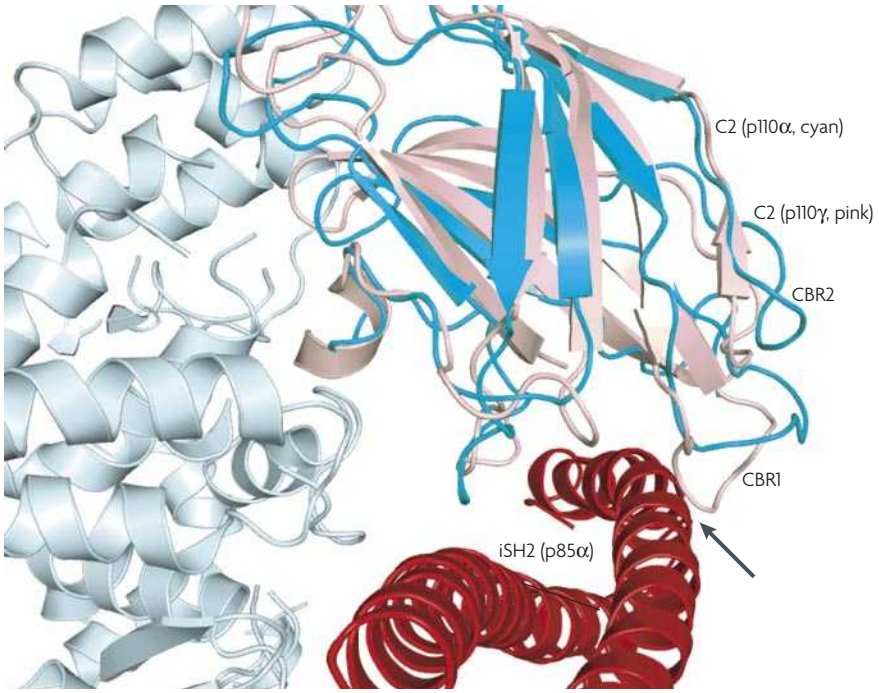

Figure 2 | **Comparison between the C2 domains of p110α and p110γ**. The C2 domains from p110α (cyan) and p110γ (pink) are aligned. The incompatibility between calcium binding region 1 (CBR1) of p110γ and inter SH2 domain (iSH2) binding is indicated by an arrow (see text).

subunit p101, but substantial differences exist between the conformations of the CBR1 loops of p110α and p110γ: when the structures of p110γ and p110α–niSH2 are aligned, the Cα carbons of some residues in the CBR1 loop of p110γ (residues 370–379) and that of p110α are >7 Å apart. In fact, the conformation of the CBR1 loop of p110γ is incompatible with binding to p85 because it would clash with the iSH2 (FIG. 2). This explains why the catalytic subunit p110γ uses an entirely different regulatory protein (p101). Conversely, we predict that the C2 domains of the other class IA catalytic subunits, p110β and p110δ, will be found to interact with the iSH2 domains of p85 using the same contacts as p110α. In p110α–niSH2, Asn345 of C2 is within hydrogen bonding distance of Asn564 and Asp560 in the iSH2 coiled coil of the p85 regulatory subunit. Although the overall sequence identity among the C2 domains of the class IA PI3Ks is relatively low (~36%), Asn345 in p110α is conserved in p110β (Asn344) and p110δ (Asn334) (see Supplementary information S1 (figure)). In addition, Asn564 and Asp560 in p85α are conserved in the regulatory subunits of class IA PI3Ks (p85β and p55γ). Thus, this hydrogen bond appears to mediate a conserved interaction between the catalytic and the regulatory subunits in all class IA PI3Ks.

Helical domain. Two mutations, Glu542Lys and Glu545Lys, in the helical domain of p110α occur with high frequency in cancers. Although Glu545 is conserved in all class IA PI3Ks, the corresponding residue is alanine in p110γ (see Supplementary information S1 (figure)). Both Glu542 and Glu545 occur at the interface between the helical domain of p110α and the nSH2 domain of p85α (REFS 25,26). Furthermore, this contact is in a region of nSH2 that also makes contact with the kinase domain of p110α. These mutations were therefore hypothesized to alter the contact between the helical domain and nSH2 in such a way that the presence of nSH2 no longer inhibits the kinase activity. This release of the nSH2 inhibition is thus equivalent to that produced by binding phosphorylated peptide to nSH2. This interpretation is supported by the recent experiments of Carson *et al.*[31], which showed that helical domain mutations increase the activity of PI3Kα by a factor of 2–4 but that the activity is not further increased by tyrosine-phosphorylated peptides that normally activate the wild-type enzyme.

Kinase domain (residues 697–1068 of p110α and 726–1102 of p110γ). The kinase domains of p110α and p110γ contain most of the conserved residues in the two proteins. It is therefore striking that the positions of two equivalent helices in these domains, helix αK12 in p110α (residues 1032–1048) and helix kα11 in p110γ (residues 1064–1078), constitute one of the most divergent features of the two structures (root mean square distance 3.2 Å; FIG. 3a). Furthermore, the residues following the αK12 helix are disordered in the p110α–niSH2 structure, whereas the equivalent residues (1081–1090) form a short helix at the end of the p110γ structure (FIG. 3a). These differences might be of mechanistic importance. The αK12 helix is spatially close to the activation loop (residues 933–957) of p110α, which determines the substrate specificity[32,33] and possibly the activation status of PI3Ks. In addition, the nSH2 domain of p85α, which was shown to inhibit the activity of PI3Kα (REF. 30), was tentatively placed in a region close to αK12 of p110α on the basis of weak electron density. We hypothesize that the position of αK12 in p110α, which is influenced by the nSH2 domain and possibly by other factors, regulates enzyme activity through its effect on the activation loop.

His1047Arg oncogenic mutant of p110α

Histidine (His)1047Arg, in helix αK12 of p110α, is one of the two most frequently observed oncogenic mutations in p110α. Interestingly, the residue corresponding to 1047 of p110α is Arg1076 in p110γ. In the p110α–niSH2 complex, His1047 is within hydrogen bonding distance of the main-chain carbonyl of leucine (Leu) 956 (FIG. 3b), which corresponds to Leu987 of p110γ and is therefore conserved between α and γ. In the p110γ structure, however, no interaction between Arg1076 and Leu987 is observed. Instead, Arg1076 is within hydrogen bonding distance of the main-chain carbonyl of Lys1000 (FIG. 3c). This hydrogen bonding shift results in the movement of helix kα11 away from the activation loop in p110γ. The change places the C-terminal end of the activation loop of p110γ in a conformation that is more open than that in p110α (FIG. 3d). The oncogenic His1047Arg mutation in p110α could lead to the formation of a new hydrogen bond involving the Arg residue, resulting in the movement of αK12 to a position similar to that of kα11 in p110γ and allowing easier access of substrates to the catalytic site. It is known that nSH2 inhibits the activity of p110α (REF. 30) and that the His1047Arg mutation increases p110α activity[17,34,35]. These observations suggest that the positions of αK12 and the activation loop observed in the p110α–niSH2 structure correspond to those of the inhibited state and that the positions of kα11 and the activation loop in the p110γ structure correspond to the activated state.

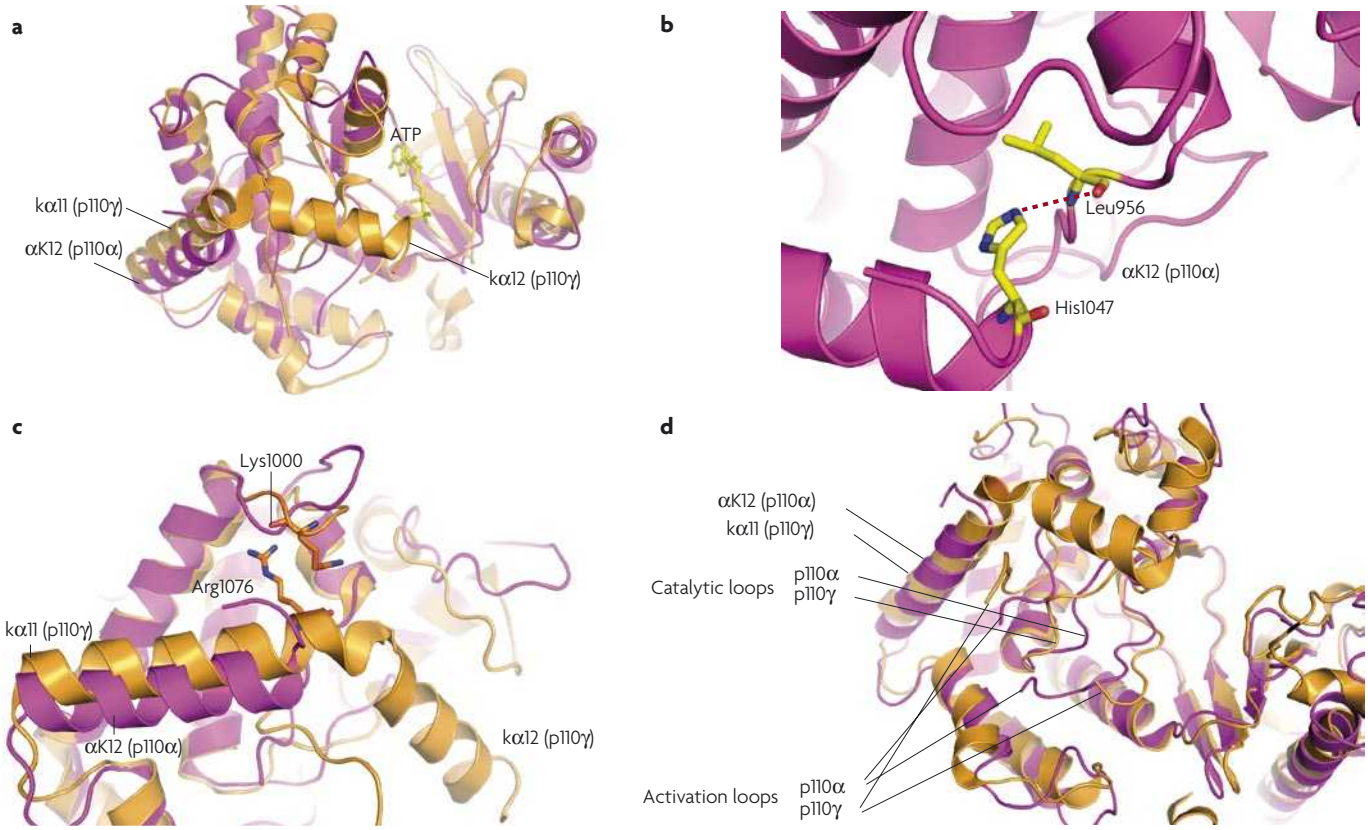

Figure 3 | **Comparison between the kinase domains of p110α and p110γ. a** | The two equivalent helices, αK12 of p110α and kα11 of p110γ, are shown in magenta and orange, respectively. The positions of the helices in each of the structures are shown by the arrows. **b** | The interaction between histidine (His) 1047 and leucine (Leu) 956 in p110α is shown. **c** | The interaction between arginine (Arg) 1076 and lysine (Lys) 1000 in p110γ is shown. Superposition of the p110α shows the different position of αK12 helix compared with kα11 helix of p110γ. **d** | Conformations of the activation and catalytic loops of p110α and p110γ.

Accordingly, p110γ can be regarded as a naturally occurring His1047Arg mutant.

Insights into inhibitor selectivity

As noted above, the ATP binding pocket in the p110α–niSH2 crystal is occupied by a loop of the RBD from a neighbouring molecule. As most PI3K inhibitors interact with the ATP binding pocket, it is not possible to obtain inhibitor-bound crystals in the same crystal form. Nevertheless, structures of several inhibitors bound to p110γ have been reported[23,36], and comparisons between the active site conformations of p110α–niSH2 and inhibitor-bound p110γ can provide insights into the basis for selectivity.

The residues that line the ATP binding pockets of p110α and p110γ are highly conserved and have similar three-dimensional structures, suggesting that inhibitors bind to these two PI3K isoforms in a similar manner (see Supplementary information S2 (figure) part a). For example, wortmannin forms a covalent bond with Lys833 and makes hydrogen bonds with Asp964, Ile963, Val882 and serine 806 in p110γ (REF. 23). All five of these residues are conserved in p110α and show little deviation of Cα carbons (0.6~1.8 Å) when the two structures are aligned (see Supplementary information S2 (figure) part a). Only slight movements of the active site loops would be required for p110α to bind wortmannin in a mode identical to that of p110γ. However, the loop between residues 771 and 779 (IMSSAKRPL) in p110α adopts a different conformation than the corresponding loop in p110γ (VMASKKKPL, residues 803–811). This difference in loop conformations is not the result of a change of p110γ induced by inhibitor binding, as the free p110γ (REF. 23) adopts a loop conformation similar to the inhibitor-bound p110γ rather than that of p110α–niSH2 (see Supplementary information S2 (figure) part c).

One inhibitor that shows selectivity for the various PI3K enzymes is the quinazolinone purine PIK-39 (REF. 36). The structure of PIK-39 bound to p110γ, determined by X-ray diffraction[36], shows that the quinazolinone moiety of PIK-39 extends perpendicular to the plane in which the aromatic moieties of most PI3K inhibitors reside, causing a conformational change of the side chain of methionine (Met) 804. Modeling of PIK-39 in the binding site of p110α places the Cα and Cβ of Met772 in p110α (Met772 of p110α corresponds to Met804 in p110γ) at only 2.5 Å and 1.1 Å away from the tip of the quinazolinone of PIK-39. This proximity is the result of the different conformation of the loop containing residues 771–779 in p110α that is noted above. It would therefore be difficult for p110α to accommodate PIK-39, even with a conformational change of the Met772 side chain (see Supplementary information S2 (figure) part d). This observation suggests that the different conformations of the loops containing residues 771–779 could be exploited for the design of isoform-specific inhibitors. Indeed, the conformation of Met804 in PI3Kγ has been proposed as a possible target of isoform-specific inhibitors[36].

Summary and conclusions

The availability of the structure of the p110α–niSH2 complex allows a detailed comparison of class IA PI3Ks as well as comparisons between class IA and class IB PI3Ks, revealing features that distinguish the two subclasses of enzymes and providing mechanistic insights into the regulation of class I PI3K activities. Importantly, a crucial helix in the kinase domain appears to regulate the catalytic activity in both physiological and pathological conditions.

Because PI3Kα is an enzyme that is frequently activated by mutations in cancers, it is regarded as a promising target for anticancer therapeutics. One current challenge is the development of isoform-specific inhibitors that would presumably minimize adverse effects. Despite the high degree of conservation of the enzyme active site, the structures of p110α–niSH2 and p110γ show significant differences in the conformation of a loop, which explains the selectivity of at least one class of inhibitor. This structural feature might provide a basis for the design and optimization of isoform-specific PI3K inhibitors in the future.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BAD | caspase 9 | *CDKN1A* | CHUK | FRAP1 | GSK3β | MDM2 | PDK1 | *PIK3CA* | *PIK3CB* | *PIK3CD* | *PIK3CG* | *PIK3R1* | *PIK3R2* | *PIK3R3* | TSC2

National Cancer Institute: http://www.cancer.gov/
brain tumour | breast cancer | colon cancer | liver cancer | ovarian cancer

FURTHER INFORMATION

L. Mario Amzel's homepage:
http://biophysics.med.jhmi.edu/amzelm/amzel.html

SUPPLEMENTARY INFORMATION

See online article: S1 (figure) | S2 (figure)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF