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Emerging common themes in regulation of PIKKs and PI3Ks

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Phosphatidylinositol-3 kinase-related kinases (PIKKs) comprise a family of protein kinases that respond to various stresses, including DNA damage, blocks in DNA replication, availability of nutrients and errors in mRNA splicing. PIKKs are characterized by the presence of a conserved kinase domain (KD), whose activity is regulated by two C-terminal regions, referred to as PIKK-regulatory domain (PRD) and FRAP-ATM-TRRAP-C-terminal (FATC), respectively. Here, we review functional and structural data that implicate the PRD and FATC domains in regulation of PIKK activity, drawing parallels to phosphatidylinositol-3 kinases (PI3K), lipid kinases that have sequence similarity to PIKKs. The PI3K C-terminus, which we propose to be equivalent to the PRD and FATC domains of PIKKs, is in close proximity to the activation loop of the KD, suggesting that in PIKKs, the PRD and FATC domains may regulate kinase activity by targeting the activation loop.

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Introduction

Phosphatidylinositol-3 kinase-related kinases (PIKKs) comprise a family of Ser/Thr-protein kinases with sequence similarity to phosphatidylinositol-3 kinases (PI3Ks). PIKKs are conserved in evolution and several homologues can be found in many organisms starting from yeast (Keith and Schreiber, 1995; Manning *et al*, 2002). In human beings, the PIKK family includes six members: ataxia-telangiectasia mutated (ATM), ataxia- and Rad3-related (ATR), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), mammalian target of rapamycin (mTOR), suppressor of morphogenesis in genitalia (SMG-1) and transformation/transcription domain-associated protein (TRRAP). Several of these members are linked to human diseases. Germline mutations targeting ATM, ATR and DNA-PKcs lead to ataxia-telangiectasia, Seckel syndrome and severe combined immunodeficiency, respectively (Savitsky *et al*, 1995; O'Driscoll *et al*, 2003; van der Burg *et al*, 2009). Further, somatic mutations targeting ATM are frequent in lymphoma, colon cancer and lung adenocarcinoma (Fang *et al*, 2003; Greenman *et al*, 2007; Ding *et al*, 2008).

At the cellular level, PIKKs have diverse biological functions. ATM, ATR and DNA-PKcs are involved in the response to DNA damage: ATM and DNA-PKcs respond to DNA double-strand breaks (DSBs) and ATR to DNA replication blocks or other conditions that lead to formation of long stretches of single-stranded DNA (Shiloh, 2003). mTOR is a nutrient-regulated kinase that controls a wide variety of pathways involved in metabolism and cell growth (Wullschleger *et al*, 2006). SMG-1 is part of the mRNA surveillance complex that regulates nonsense-mediated mRNA decay (Yamashita *et al*, 2005). Finally, TRRAP functions as part of a multiprotein co-activator complex possessing histone acetyltranferase activity that is important for the transcriptional activity of c-Myc and other transcription factors (McMahon *et al*, 2000). It should be noted that TRRAP, unlike the other PIKKs, does not possess kinase activity, because the amino acids required for such activity are absent in its kinase-like domain (McMahon *et al*, 1998). Nevertheless, TRRAP has high sequence similarity with the other PIKKs and is considered as a true member of this family.

At the protein level, four domains are conserved in PIKKs and distinguish them from other protein kinases (Figure 1A). From the N-terminus to the C-terminus, these are the FRAP-ATM-TRRAP (FAT) domain, the kinase domain (KD), the PIKK-regulatory domain (PRD) and the FAT-C-terminal (FATC) domain (Keith and Schreiber, 1995; Bosotti *et al*, 2000; Mordes *et al*, 2008). The KD has low sequence similarity to classical eukaryotic protein kinases, which is why PIKKs are considered as atypical protein kinases (Manning *et al*, 2002). In fact, the KDs of PIKKs have higher sequence similarity to PI3Ks, lipid kinases that phosphorylate the 3-hydroxyl group of phosphoinositides to generate second messengers that induce cell proliferation (Cantley, 2002). Owing to this sequence similarity, PI3Ks may serve as a framework for understanding PIKK function.

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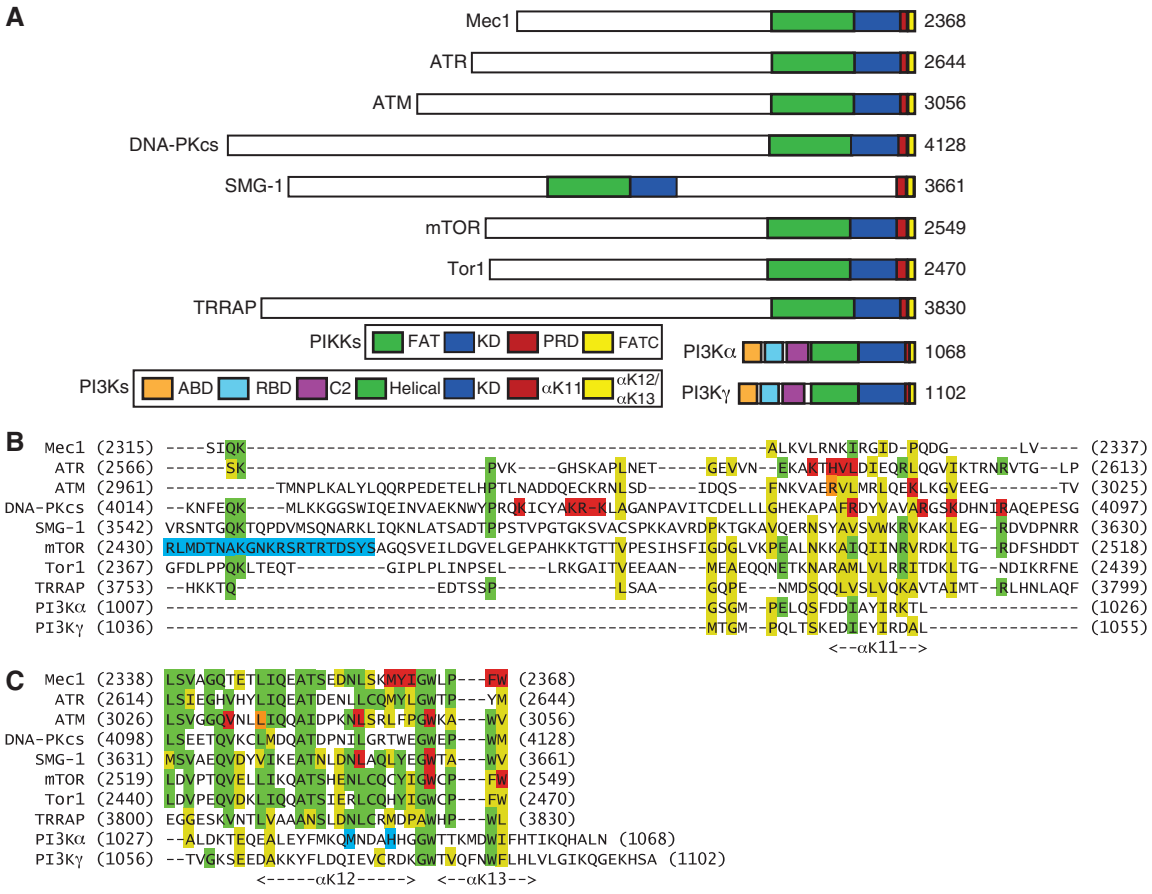

Figure 1 Domain structure and alignment of PIKKs and PI3Ks. (**A**) Schematic presentation of the known protein domains of selected PIKKs and PI3Ks. All the kinases shown are human, except for mTOR, which is of rat origin, and Mec1 and Tor1, which are *S. cerevisiae* proteins. PI3Kα and PI3Kγ refer to the PI3K catalytic subunits p110α and p110γ, respectively. The abbreviations of the domains are mentioned in the text. (B, C) Amino-acid alignment of the PRD (**B**) and FATC (**C**) domains of selected PIKKs to the C-terminal α-helices of the PI3K catalytic subunits p110α and p110γ. The boundaries of helices αK11, αK12 and αK13 of p110γ are shown below its sequence. Regions of homology are coloured green (four or more identical residues at that position) and yellow (five or more similar residues at that position). Residues, whose substitution decreases kinase activity, are coloured red; residues, whose substitution does not affect or enhances kinase activity, are coloured blue; residues of ATM targeted naturally by mutations in ataxia-telangiectasia patients are coloured orange. Residue numbers for each protein are indicated in parentheses.

Mammalian PI3Ks are divided into three classes designated by roman numerals (Domin and Waterfield, 1997). Of interest to this review, class I enzymes consist of a catalytic subunit, of which there are four isoforms and a regulatory subunit. The catalytic subunits p110α, p110β and p110δ associate with the SH2-domain containing regulatory subunit p85, whereas the catalytic subunit p110γ associates with the regulatory subunit p101, which interacts with G-protein-coupled receptors (Carpenter *et al*, 1993; Stephens *et al*, 1997). The gene encoding the p110α catalytic subunit is mutated in many human cancers with the frequency of mutation reaching 30% in colon and breast cancer (Samuels *et al*, 2004; Vogt *et al*, 2007; Zhao and Vogt, 2008).

Regulation of PIKKs by the FAT, FATC and PRD domains

As mentioned above, three domains, referred to as FAT, PRD and FATC, are conserved in PIKKs, in addition to the KD (Bosotti *et al*, 2000) (Figure 1A). These three domains regulate the activity of the KD.

The FAT domain is just N-terminal to the KD. The three-dimensional structure of a small part of the FAT domain of mTOR has been determined and shows a domain consisting entirely of four α-helices (Choi *et al*, 1996). In mTOR, this part of the FAT domain, just N-terminal to the KD, is referred to as the FKBP12-rapamycin-binding (FRB) domain, because it binds the complex of the FKBP12 protein with rapamycin (Stan *et al*, 1994; Chen *et al*, 1995). Sequence analysis of PIKK family members suggests that the entire FAT domain and even the entire N-terminus of these proteins is α-helical (Perry and Kleckner, 2003). The sequence analysis further predicts that the majority of these α-helices adopt a tertiary structure similar to that of HEAT repeats (so called, because they are present in *H*untingtin, *E*longation factor 3, *A*lpha-regulatory subunit of protein phosphatase 2A and *T*OR1). This prediction is supported by electron microscopy-derived structures of human ATM, human DNA-PKcs and yeast TOR1, which show that their N-termini form a curved tubular-shaped domain similar to that adopted by proteins containing HEAT repeats (Llorca *et al*, 2003; Rivera-Calzada *et al*, 2005; Spagnolo *et al*, 2006; Adami *et al*, 2007).

There is strong evidence that the FAT domain and the predicted HEAT repeats N-terminal to the KD regulate PIKK kinase activity. First, the FRB domain of mTOR is bound by the complex of rapamycin with FKBP12 and rapamycin is a potent inhibitor of mTOR kinase activity (Stan *et al*, 1994; Chen *et al*, 1995; Choi *et al*, 1996). One could speculate that rapamycin competes for binding of activators to mTOR. Second, Nbs1, a subunit of a tripartite protein complex that recognizes DNA DSBs, binds to the N-terminus of ATM and enhances its kinase activity (You *et al*, 2005). Finally, Ku70/Ku80 heterodimers and DNA induce conformational changes in the FAT domain of DNA-PKcs and enhance its kinase activity (Spagnolo *et al*, 2006).

The FATC domain comprises the very C-terminus of PIKKs. It is a small, highly conserved domain of about 30 amino acids in length (Bosotti *et al*, 2000). The high degree of sequence conservation makes it possible to substitute FATC domains among certain PIKK family members and maintain functionality. For example, the FATC domain of ATM can be replaced by the FATC domains of ATR, TRRAP or DNA-PKcs without loss of function (Jiang *et al*, 2006). However, when the FATC domains of ATR or mTOR are replaced by that of ATM, then function is lost (Takahashi *et al*, 2000; Mordes *et al*, 2008).

The three-dimensional structure of the FATC domain of *Saccharomyces cerevisiae* Tor1 has been determined by NMR spectroscopy and is comprised of an α-helix followed by a sharp turn. The turn is stabilized by a disulphide bond formed between Cys2460 in the helix and Cys2467 in the sequence C-terminal to the helix (Dames *et al*, 2005). The FATC domain has also been interpreted to have been visualized in low resolution electron microscopy structures of yeast Tor1 and human DNA-PKcs; in these structures, it is shown to protrude from the KD (Rivera-Calzada *et al*, 2005; Spagnolo *et al*, 2006; Adami *et al*, 2007).

Several studies indicate that the FATC domain of PIKKs is critical for kinase activity and is very sensitive to mutagenesis. Deletion of even one residue from the C-terminus of mTOR abolishes kinase activity (Peterson *et al*, 2000) and various single and double amino-acid substitutions in the FATC domain of several PIKKs reduce kinase activity dramatically (Takahashi *et al*, 2000; Nakada *et al*, 2005; Sun *et al*, 2005; Morita *et al*, 2007). The mutagenesis experiments suggest that the conserved hydrophobic amino acids in the FATC domain are critical for function (Figure 1C). For example, in SMG-1, substitution of Leu3646 with alanine reduces kinase activity by $>90\%$ and substitution of Trp3653 with phenylalanine, a substitution that has only a small effect on hydrophobicity, reduces kinase activity by 50% (Morita *et al*, 2007). A naturally occurring mutation in a patient with ataxia-telangiectasia also targets the FATC domain of ATM (Cavalieri *et al*, 2006).

The current model for FATC domain function proposes that these domains mediate protein–protein interactions. For example, the histone acetyltransferase Tip60 binds to the FATC domains of ATM and DNA-PKcs and enhances their kinase activity in response to DNA damage. For ATM, it has further been shown that the mechanism involves direct acetylation of its PRD domain by Tip60 (Sun *et al*, 2005, 2007; Jiang *et al*, 2006). A second example involves Mec1, the budding yeast homologue of human ATR, whose FATC domain interacts with Rfa1 (Nakada *et al*, 2005). Rfa1 is the largest subunit of the yeast replication protein A (RPA) complex, a single-stranded DNA-binding protein that localizes to sites of DNA damage and stalled replication forks. The interaction of the FATC domain of Mec1 with Rfa1 not only facilitates recruitment of Mec1 to sites of DNA damage, but may also have other functional consequences, such as regulation of the kinase activity of Mec1 (Nakada *et al*, 2005). Rfa1 also binds Ddc2, a protein that associates tightly with Mec1; the Rfa1–Ddc2 interaction is conserved in human cells and seems to also be responsible for recruitment of ATR and Mec1 to sites of DNA damage and replication stress (Zou and Elledge, 2003; Ball *et al*, 2005).

The PRD was recently defined as the region between the kinase and FATC domains (Mordes *et al*, 2008). This region is not very highly conserved between different PIKKs and, with the exception of SMG-1, its length varies between 16 and 82 amino acids. Deletion of the entire PRD abolishes kinase activity, but specific small deletions within the PRD, for example, of residues 2430–2450 of rat mTOR or of residues 2569–2576 of human ATR do not compromise or even, in the case of mTOR, enhance kinase activity (Sekulic *et al*, 2000; Mordes *et al*, 2008). These small deletions correspond to the N-terminal half of the PRD, which shows almost no sequence conservation among PIKKs (Figure 1B). A monoclonal antibody raised against amino-acids 2433–2450 of rat mTOR also enhances kinase activity (Brunn *et al*, 1997), mimicking the effect of deleting these residues.

The more highly conserved C-terminal half of the PRD (Figure 1B) appears to be the site of posttranslational modifications or protein–protein interactions that enhance PIKK kinase activity. In ATM, Lys3016 becomes acetylated by Tip60 and substitution of this residue with arginine does not affect basal ATM kinase activity, but suppresses the activation of ATM by DNA damaging agents (Sun *et al*, 2005, 2007). Further, Arg3008 is substituted by cysteine in an ataxia-telangiectasia patient (Li and Swift, 2000). In ATR, the PRD interacts with the activation domain of topoisomerase II-binding protein 1 (TopBP1); this is the domain of TopBP1 that enhances ATR kinase activity *in vitro* and *in vivo* (Kumagai *et al*, 2006; Mordes *et al*, 2008). Specific amino-acid substitutions targeting the PRD of ATR, such as of Lys2589 with glutamic acid, compromise the interaction of ATR with TopBP1, the induction of TopBP1-dependent kinase activity and the checkpoint function of ATR, but do not affect basal kinase activity (Mordes *et al*, 2008). In DNA-PKcs, charge-reversal amino-acid substitutions targeting the PRD also compromise its kinase activity (Mordes *et al*, 2008). Finally, in rat mTOR, two residues within its PRD, Thr2446 and Ser2448, are phosphorylated by S6K1 in response to mitogens and nutrients (Chiang and Abraham, 2005; Holz and Blenis, 2005), although the function, if any, of these phosphorylations on mTOR kinase activity is not well established (Sekulic *et al*, 2000).

Regulation of PI3Ks: structure–function relationship

No atomic resolution structures of the KDs of any of the PIKKs have been determined. However, the three-dimensional structures of the PI3K catalytic subunits p110α and p110γ have been determined alone or in complex with different co-regulators. The first solved structure was that of

residues 144–1102 of p110γ with bound ATP (Walker *et al*, 1999). This was followed by the structure of the same fragment of p110γ in complex with Ras (Pacold *et al*, 2000) and then by the structure of full-length p110α in complex with a fragment of the p85α-regulatory subunit (Huang *et al*, 2007). The structure of the N-terminally truncated p110γ catalytic subunits show four domains: a Ras-binding domain (RBD), a C2 domain, a helical domain and the KD, whereas the structure of the full-length p110α subunit also shows the adaptor-binding domain (ABD) (Figure 1A). The three-dimensional structures of the helical and KDs are of greatest interest from the PIKK perspective and, therefore, only these will be described here (Figure 2).

The helical domains of p110α and p110γ adopt very similar structures consisting of pairs of anti-parallel α-helices. These pairs form a stack with an arrangement very similar to that present in HEAT repeats. Thus, even though the amino-acid sequences of the PI3K catalytic subunits do not match the motif for HEAT repeats, their three-dimensional structure reveals significant structural similarity to that of HEAT repeats (Walker *et al*, 1999). This then raises the possibility that the FAT domains of the PIKKs, which have been predicted to be HEAT repeats (Perry and Kleckner, 2003), adopt a three-dimensional structure very similar to that of the helical domains of p110α and p110γ.

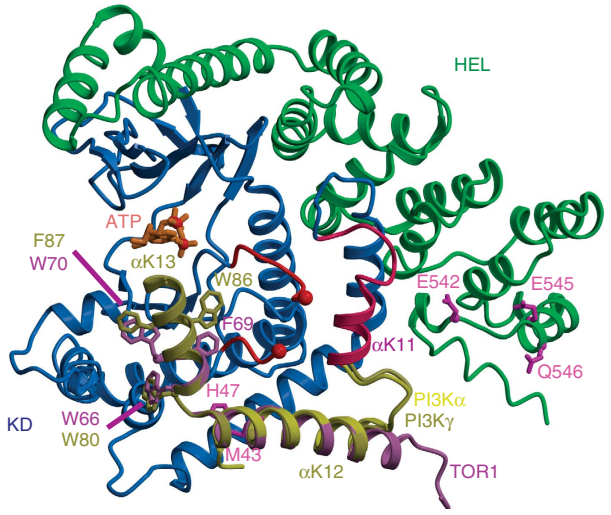

Figure 2 Superimposition of the FATC domain of *S. cerevisiae* Tor1 (PDB file 1w1n) and of helices αK12 and αK13 of the PI3K catalytic subunit p110γ (PDB file 1e8x) on the three-dimensional structure of the helical and kinase domains (KDs) of the PI3K catalytic subunit p110α (PDB file 2rd0), according to the alignment shown in Figure 1B. The p110α helical (HEL) and KDs are coloured green and blue, respectively. The activation loop is coloured red; the red spheres mark the boundaries of the part of the activation loop, whose structure was not determined. The ATP, from the p110γ structure, is coloured orange. Helices αK11 and αK12 of p110α are coloured purple and bright yellow, respectively. Helix αK13 of p110α was not resolved in the electron density map and is not shown. Helices αK12 and αK13 of p110γ are coloured dark yellow, as are the side chains of the p110γ residues Trp1080 (W80), Trp1086 (W86) and Phe1087 (F87). The FATC domain of Tor1 is coloured light purple, as are the side chains of its residues Trp2466 (W66), Phe2469 (F69) and Trp2470 (W70). Note that residues 2465–2470 of Tor1 were modelled according to the p110γ structure, as described in the text. Residues of p110α targeted by cancer-associated mutations map to the N-terminus of the helical domain (E542, Glu542; E545, Glu545; Q546, Gln546) and to helix αK12 (M43, Met1043; H47, His1047). PI3Kα and PI3Kγ refer to the PI3K catalytic subunits p110α and p110γ, respectively.

In PI3Ks, the helical domains serve as a scaffold to which the other domains of the catalytic subunit are attached (Figure 2). Three residues towards the N-terminus of the helical domain of p110α, Glu542, Glu545 and Gln546 are frequently targeted by mutations found in human cancers (Samuels *et al*, 2004; Gymnopoulos *et al*, 2007). The tumour-associated mutations at these positions are charge-reversal mutations (glutamic acid to lysine) that enhance kinase activity and make p110α insensitive to regulation by p85α (Ikenoue *et al*, 2005; Isakoff *et al*, 2005; Kang *et al*, 2005; Samuels *et al*, 2005; Zhao *et al*, 2005; Bader *et al*, 2006; Gymnopoulos *et al*, 2007; Zhao and Vogt, 2008). In the wild-type p110α/p85α complex, the N-terminal SH2 (nSH2) domain of p85α binds to the negatively charged N-terminus of the helical domain of p110α; this interaction, which brings the nSH2 domain close to the KD, suppresses kinase activity (Yu *et al*, 1998a, b; Miled *et al*, 2007). Under growth-stimulating conditions, negatively charged tyrosine-phosphorylated receptors bind to the nSH2 domain of p85α and prevent it from interacting with the p110α subunit, thus relieving its inhibitory effect on the kinase. According to this model, the tumour-associated mutations have a similar effect, displacing the nSH2 domain from the p110α subunit (Miled *et al*, 2007).

The KDs of p110α and p110γ adopt very similar three-dimensional structures to one another and interact extensively with the helical domain (Figure 2). Despite the poor sequence similarity between the KDs of PI3Ks and classical protein kinases, at the three-dimensional level there are considerable similarities. In both families, the KDs have N- and C-terminal lobes and conserved secondary structure elements, especially around the ATP-binding site. An interesting feature in the PI3K structures is the activation loop, a significant part of which is disordered and, therefore, not visible in the electron density maps. However, the N- and C-terminal ends of the activation loop are visible and are surrounded from three sides by the three C-terminal α-helices of the KD. These helices are better resolved in the structure of p110γ bound to ATP. We will refer to them as helices αK11, αK12 and αK13 (Figure 2), so that we have the same terminology for both p110α and p110γ, although in p110γ they have been referred to as helices αK10, αK11 and αK12, respectively (Walker *et al*, 1999; Pacold *et al*, 2000). These three helices are on the same plane and form the three sides of an imaginary rectangle. The N- and C-terminal ends of the activation loop are within this rectangle. The most C-terminal helix, αK13, is characterized by the presence of conserved residues with large hydrophobic side chains, such as Trp1080, Trp1086 and Phe1087. The side chains of these residues face towards the hydrophobic core of the KD and are, therefore, likely to stabilize the position of helix αK13 against the rest of the KD. Interestingly, in the p110α structure, the C-terminal α-helix is disordered and, apparently, not in contact with the rest of the KD (Huang *et al*, 2007). This raises the possibility that the C-terminal helix of the KD of PI3Ks is conformationally flexible, perhaps adopting different conformations in the active and inactive states.

Tumour-associated mutations targeting residues in the KD of p110α are quite frequent and, interestingly, map to the C-terminal half of helix αK12 (Samuels *et al*, 2004; Gymnopoulos *et al*, 2007). The targeted residues are His1047, which is substituted with arginine, leucine or tyrosine, and Met1043, which is substituted with isoleucine or

valine (Figure 2). As His1047 and Met1043 face the core of the KD, their substitution may affect the interaction of helix αK12 and, by consequence, of helix αK13 with the rest of the KD. The proximity of helices αK12 and αK13 to the activation loop suggests an effect on the conformation of the activation loop. Comparison of the structures of p110γ on its own or in complex with Ras shows an interaction of Ras with the C-terminal lobe of the KD and modest conformational changes that also affect helices αK12 and αK13 (Pacold *et al*, 2000). Exactly how these conformational changes enhance kinase activity is not clear, especially as the activation loop is disordered in both structures. However, it is of interest that the tumour-associated mutations that map to helix αK12 enhance kinase activity and render PI3K insensitive to regulation by Ras, whereas preserving regulation by p85α (Ikenoue *et al*, 2005; Isakoff *et al*, 2005; Kang *et al*, 2005; Samuels *et al*, 2005; Zhao *et al*, 2005; Bader *et al*, 2006; Gymnopoulos *et al*, 2007; Zhao and Vogt, 2008).

A model for regulation of the PIKKs

To gain more insights into the regulation of PIKKs, we considered the possibility that the three-dimensional structure of the helical and KDs of the PI3K catalytic subunits is similar to the structure of the FAT, kinase, PRD and FATC domains of PIKKs. First, as mentioned above, the helical domain of PI3Ks has a three-dimensional structure similar to that of HEAT repeats, and the amino-acid sequence of the FAT domain of PIKKs matches the HEAT repeat consensus motif (Walker *et al*, 1999; Perry and Kleckner, 2003). One may, therefore, predict that in PIKKs, the FAT domain adopts a conformation similar to that of the helical domain of PI3Ks. Further, the KDs of PIKKs and PI3Ks have significant sequence similarity and, therefore, are likely to adopt similar three-dimensional structures. Finally, the three C-terminal α-helices of the KD of p110γ have sequence similarity to the PRD and FATC domains of PIKKs: helix αK11 of p110γ can be aligned to the C-terminal half of the PRD domain, whereas helices αK12 and αK13 can be aligned to the FATC domain (Figure 1B and C). If indeed, the PRD and FATC domains of PIKKs adopt a three-dimensional structure similar to that of the C-terminal three α-helices of p110γ, then they would be physically close to the activation loop and, thus, ideally placed to regulate kinase activity.

The only PIKK polypeptides, whose three-dimensional structure has been determined at the atomic level, are the FRB domain of mTor and the FATC domain of budding yeast Tor1 (Choi *et al*, 1996; Dames *et al*, 2005). We wondered whether we could superimpose the FATC domain of Tor1 on the structure of the PI3K catalytic subunit on the basis of their sequence alignment (Figure 1B). As mentioned above, the FATC domain of Tor1 folds as an α-helix followed by a sharp turn. The latter is stabilized by a disulphide bridge between Cys2460 and Cys2467 (Dames *et al*, 2005). However, these two cysteines are not conserved in other PIKKs and are unlikely to form a disulphide bond in the reducing intracellular environment. Therefore, we superimposed the α-helix of Tor1 on helix αK12 of p110γ, but then modelled the remaining FATC residues according to the p110γ structure. Doing so led to the conserved C-terminal Tor1 residues Trp2466, Phe2469 and Trp2470 facing towards the KD, in a manner analogous to the orientation of the p110γ conserved residues Trp1080, Trp1086 and Phe1087 (Figure 2).

The possibility that the FAT, kinase, PRD and FATC domains of PIKKs adopt a similar three-dimensional structure to the helical and KDs of PI3Ks provides some room for speculation on how PIKK kinase activity may be regulated. In PI3Ks, activation requires two converging mechanisms (Miled *et al*, 2007). The first mechanism involves activated Ras, whose binding to the RBD of PI3Ks induces conformational changes in the C-terminal lobe of the KD (Pacold *et al*, 2000). These conformational changes may facilitate enhancement of the kinase activity by targeting the αK12 and αK13 helices, since amino-acid substitutions associated with cancer that target these helices enhance kinase activity and render it insensitive to regulation by Ras. The second mechanism involves the nSH2 domain of the p85α-regulatory subunit. This SH2 domain binds to the helical domain when PI3K is inactive, but is released from the helical domain when a competing SH2-binding site becomes available in growth factor-stimulated cells; these competing SH2-binding sites are phosphorylated tyrosines of activated growth factor tyrosine kinase receptors (Miled *et al*, 2007). The release of the SH2 domain from the helical domain and the activated Ras-induced conformational changes may synergistically impact on the conformation of the C-terminal helices αK11, αK12 and αK13, which in turn may switch on kinase activity by affecting the conformation of the PI3K activation loop.

Using the PI3K analogy, one can hypothesize that the PRD and FATC domains of PIKKs integrate different signals leading to activation of these kinases. For example, the activation domain of TopBP1 binds to the PRD of ATR (Mordes *et al*, 2008), whereas RPA binds to its FATC domain (based on evidence with the homologous yeast proteins, as described above; Nakada *et al*, 2005). Together, these interactions may switch on ATR kinase activity by synergistically affecting the conformation of the activation loop. In the case of ATM, binding of the MRN complex to its N-terminus (You *et al*, 2005) may affect the conformation of its FATC domain, somewhat analogous to how Ras affects the conformation of the C-terminal lobe of the PI3K KD, whereas Tip60 targets the PRD for acetylation (Sun *et al*, 2005, 2007). Such a model, whereby the PRD and FATC domains integrate different signals, may help explain why activation of PIKKs is not promiscuous.

Significant progress has been made regarding our understanding of the regulation of PIKKs in the last few years, but much more needs to be learnt. The determination of the three-dimensional structure of a PIKK will establish whether its PRD and FATC domains adopt a three-dimensional structure similar to that of the C-terminal α-helices of PI3Ks, as proposed here, and, accordingly, to what extent we can use insights from PI3Ks to understand PIKK function.

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Conflict of interest

The authors declare that they have no conflict of interest.

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