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 acetyltransferase 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.
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 Huntingtin, Elongation factor 3, Alpha-regulatory subunit of protein phosphatase 2A and TOR1). 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 (Cavallieri 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, Arg5008 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 p110\(\gamma\) with bound ATP (Walker et al., 1999). This was followed by the structure of the same fragment of p110\(\gamma\) in complex with Ras (Pacold et al., 2000) and then by the structure of full-length p110\(\alpha\) in complex with a fragment of the p85\(\alpha\)-regulatory subunit (Huang et al., 2007). The structure of the N-terminally truncated p110\(\gamma\) 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 p110\(\alpha\) 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 p110\(\alpha\) and p110\(\gamma\) adopt very similar structures consisting of pairs of anti-parallel \(\alpha\)-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 p110\(\alpha\) and p110\(\gamma\).

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 p110\(\alpha\), 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 p110\(\alpha\) insensitive to regulation by p85\(\alpha\) (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 p110\(\alpha\)/p85\(\alpha\) complex, the N-terminal SH2 (nSH2) domain of p85\(\alpha\) binds to the negatively charged N-terminus of the helical domain of p110\(\alpha\); 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 p85\(\alpha\) and prevent it from interacting with the p110\(\alpha\) 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 p110\(\alpha\) subunit (Miled et al., 2007).

The KDs of p110\(\alpha\) and p110\(\gamma\) 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 \(\alpha\)-helices of the KD. These helices are better resolved in the structure of p110\(\gamma\) bound to ATP. We will refer to them as helices \(\alpha\)K11, \(\alpha\)K12 and \(\alpha\)K13 (Figure 2), so that we have the same terminology for both p110\(\alpha\) and p110\(\gamma\), although in p110\(\gamma\) they have been referred to as helices \(\alpha\)K10, \(\alpha\)K11 and \(\alpha\)K12, 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, \(\alpha\)K13, 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 \(\alpha\)K13 against the rest of the KD. Interestingly, in the p110\(\alpha\) structure, the C-terminal \(\alpha\)-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 p110\(\alpha\) are quite frequent and, interestingly, map to the C-terminal half of helix \(\alpha\)K12 (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

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**Figure 2** Superimposition of the FATC domain of *S. cerevisiae* Tor1 (PDB file 1w1n) and of helices \(\alpha\)K12 and \(\alpha\)K13 of the PI3K catalytic subunit p110\(\gamma\) (PDB file 1e8x) on the three-dimensional structure of the helical and kinase domains (KD) of the PI3K catalytic subunit p110\(\alpha\) (PDB file 2rd0), according to the alignment shown in Figure 1B. The p110\(\alpha\) 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 p110\(\alpha\) structure, is coloured orange. Helices \(\alpha\)K11 and \(\alpha\)K12 of p110\(\alpha\) are coloured purple and bright yellow, respectively. Helix \(\alpha\)K13 of p110\(\gamma\) was not resolved in the electron density map and is not shown. Helices \(\alpha\)K12 and \(\alpha\)K13 of p110\(\gamma\) are coloured dark yellow, 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 p110\(\alpha\) structure, as described in the text. Residues of p110\(\alpha\) targeted by cancer-associated mutations map to the N-terminus of the helical domain (E542, Glu542, E545, Glu545, Q546, Gln546) and to helix \(\alpha\)K12 (M43, Met1043; H47, His1047). PI3K\(\alpha\) and PI3K\(\gamma\) refer to the PI3K catalytic subunits p110\(\alpha\) and p110\(\gamma\), respectively.
valine (Figure 2). As His1047 and Met1043 face the core of the KD, their substitution may affect the interaction of helix zK12 and, by consequence, of helix zK13 with the rest of the KD. The proximity of helices zK12 and zK13 to the activation loop suggests an effect on the conformation of the activation loop. Comparison of the structures of p110\gamma 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 zK12 and zK13 (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 zK12 enhance kinase activity and render PI3K insensitive to regulation by Ras, whereas preserving regulation by p85\alpha (Ikenoue et al., 2005; Isakov et al., 2005; Kang et al., 2005; Samuels et al., 2005; Zhao et al., 2005; Bader et al., 2006; Gymnopoulous 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 \(\alpha\)-helices of the KD of p110\gamma have sequence similarity to the PRD and FATC domains of PIKKs: helix zK11 of p110\gamma can be aligned to the C-terminal half of the PRD domain, whereas helices zK12 and zK13 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 \(\alpha\)-helices of p110\gamma, 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 \(\alpha\)-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 \(\alpha\)-helix of Tor1 on helix zK12 of p110\gamma, but then modelled the remaining FATC residues according to the p110\gamma 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 p110\gamma 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 zK12 and zK13 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 p85\alpha-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 phospho-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 zK11, zK12 and zK13, 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 \(\alpha\)-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.
References

Adami A, Garcia-Alvarez B, Arias-Palomo E, Barford D, Llorca O (2007) Structure of TOR and its complex with KOG1. *Mol Cell* **27**: 509–516

Bader AG, Kang S, Vogt PK (2006) Cancer-specific mutations in PIK3CA are oncogenic in *vivo*. *Proc Natl Acad Sci USA* **103**: 1475–1479

Ball HL, Myers JS, Cortez D (2005) ATRIP binding to replication protein A-single-stranded DNA promotes ATR-ATRIP localization but is dispensable for Chk1 phosphorylation. *Mol Biol Cell* **16**: 2372–2381

Bosotti R, Isacchi A, Sonnhammer EL (2000) FAT: a novel domain in PIK-related kinases. *Trends Biochem Sci* **25**: 225–227

Brunn GJ, Fadden P, Haystead TA, Lawrence Jr JC (1997) The mammalian target of rapamycin phosphorylates sites having a (Ser/Thr)-Pro motif and is activated by antibodies to a region near its COOH terminus. *J Biol Chem* **272**: 32547–32550

Cantley LC (2002) The phosphoinositide 3-kinase pathway. *Science* **296**: 1655–1657

Carpenter CL, Auger KR, Chanudhuri M, Yoakim M, Schaffhausen B, Shoeholtz S, Cantley LC (1993) Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. *J Biol Chem* **268**: 9478–9485

Cavaliere S, Funaro A, Porcedda P, Turinetto V, Migone N, Gatti RA, Brusco A (2006) ATM mutations in Italian families with ataxia telangiectasia include two distinct large genomic deletions. *Hum Mutat* **27**: 1061

Chen J, Zheng Y, Brown EJ, Schreiber SL (1995) Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. *Proc Natl Acad Sci USA* **92**: 4947–4951

Chiang GG, Abraham RT (2005) Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem* **280**: 25485–25490

Choi J, Chen J, Schreiber SL, Clardy J (1996) Structure of the FATC domain of the protein kinase of *S. cerevisiae*. *FEBS Lett* **339**: 239–242

Dames SA, Mulet JM, Rathgeb-Szabo K, Hall MN, Grzesiek S (2005) The solution structure of the FACC domain of the protein kinase target of rapamycin (mTOR) suggests a role for rapamycin-dependent structural and cellular stability. *J Biol Chem* **280**: 20558–20564

Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, Fulton L, Fulton RS, Zhang Q, Wendl MC, Lawrence MS, Larson DE, Chen K, Dooling DJ, Sabo A, Hawes AC et al (2008) Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **455**: 1069–1075

Djoumbo LE, Brown EJ, Schreiber SL (1995) Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. *Proc Natl Acad Sci USA* **92**: 4947–4951

Distant N, and terminal domains are required for intrinsic kinase activity of SMG-1, a critical component of nonsense-mediated mRNA decay. *J Biol Chem* **282**: 7799–7808

Isakoff SJ, Engelman JA, Irie HY, Luo J, Brachmann SM, Pearlve RV, Cantley LC, Brugge JS (2005) Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res* **65**: 10992–11000

Jiang X, Sun Y, Chen S, Roy K, Price BD (2006) The FATC domains of PIK3C proteins are functionally equivalent and participate in the Tip60-dependent activation of DNA-PKcs and ATM. *J Biol Chem* **281**: 15741–15746

Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol-3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* **102**: 802–807

Keith CT, Schreiber SL (1995) PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. *Science* **270**: 50–51

Kumagai A, Lee J, Yoo HY, Dunphy WG (2006) TopBP1 activates the ATR-ATRIP complex. *Cell* **124**: 945–955

Li A, Swift M (2000) Mutations at the ataxia-telangiectasia locus and clinical phenotypes of A-T patients. *Am J Med Genet* **92**: 170–177

Llorca O, Rivera-Calzada A, Grantham J, Willison KR (2003) Electron microscopy and 3D reconstructions reveal that human ATM kinase uses an arm-like domain to clamp around double-stranded DNA. *Oncogene* **22**: 3867–3874

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. *Science* **298**: 1912–1934

McMahon SB, Van Buskirk HA, Dugan KA, Copeland TD, Cole MD (1998) The novel ATM-related protein TRRAP is an essential cofactor for the C-myc and E2F oncogenes. *Cell* **94**: 361–374

McMahon SB, Wood MA, Cole MD (2000) The essential cofactor TRRAP recruits the histone acetytransferase hGCN5 to c-Myc. *Mol Cell Biol* **20**: 556–562

Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, Schneidman-Duhovny D, Wolfson HJ, Backer JM, Williams RL (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science* **317**: 239–242

Mordes DA, Glick GG, Zhao R, Cortez D (2008) TopBP1 activates ATR through ATRIP and a PIKK regulatory domain. *Genes Dev* **22**: 1478–1489

Morita T, Yamashita A, Kashima I, Ogata S, Ishiura S, Ohno S (2007) The PIKK proteins are functionally equivalent and participate in the ataxia-telangiectasia locus and Rad3-related protein (ATR) mutants results in Seckel syndrome. *Nat Genet* **39**: 497–501

Nakada D, Hirano Y, Tanaka Y, Sugimoto K (2005) Role of the C terminus of Mecl1 checkpoint kinase in its localization to sites of DNA damage. *Mol Biol Cell* **16**: 5227–5235

O'Driscoll M, Ruiz-Perez V, Woods CG, Jorgo PA, Goodship JA (2003) A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. *Nat Genet* **39**: 497–501

Pacold ME, Suire S, Perisic O, Lara-Gonzalez S, Davis CT, Walker EH, Hawkins FT, Stephens L, Eccleston JF, Williams RL (2000) Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. *Cell* **103**: 931–943

Perry J, Kleckner N (2003) The ATRs, ATMs, and TORs are giant effector phosphoinositide 3-kinase-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. *J Biol Chem* **275**: 7416–7423

Rivera-Calzada A, Maman JD, Spagnolo L, Pearl LH, Llorca O (2005) Three-dimensional structure and regulation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). *Structure* **13**: 243–255

Samuels Y, Diaz Jr LA, Schmidt-Kittler O, Cummins JM, Delong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE (2005) Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Res* **65**: 2481–2488

Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* **304**: 554

Sajchut K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Friedman M, Harnik R, Patanji SI, Simmons A, Clines GA,
Regulation of PIKKs and PI3Ks
H Lempia¨inen and TD Halazonetis

Sartiel A, Gatti RA, Chessa L et al (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749–1753

Sekulic A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, Abraham RT (2000) A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. Cancer Res 60: 3504–3513

Shiloh Y (2003) ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 3: 155–168

Spagnolo L, Rivera-Calzada A, Pearl LH, Llorca O (2006) Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. Mol Cell 22: 511–519

Stan R, McLaughlin MM, Cafferkey R, Johnson RK, Rosenberg M, Livi GP (1994) Interaction between FKBP12-rapamycin and TOR involves a conserved serine residue. J Biol Chem 269: 32027–32030

Stephens LR, Eguinoa A, Erdjument-Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. Cell 89: 105–114

Sun Y, Xu Y, Roy K, Price BD (2007) DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. Mol Cell Biol 27: 8502–8509

Takahashi T, Hara K, Inoue H, Kawa Y, Tokunaga C, Hidayat S, Yoshino K, Kuroda Y, Yonezawa K (2000) Carboxyl-terminal region conserved among phosphoinositide-kinase-related kinases is indispensable for mTOR function in vivo and in vitro. Genes Cells 5: 765–775

van der Burg M, Ijspeert H, Verkaai NS, Turul T, Wiegant WW, Morotomi-Yano K, Mari PO, Tezcan I, Chen DJ, Zdziennicka MZ, van Dongen JJ, van Gent DC (2009) A DNA-PKcs mutation in a radiosensitive T-B- SCID patient inhibits Artemis activation and nonhomologous end-joining. J Clin Invest 119: 91–98

Vogt PK, Kang S, Elsliger MA, Gymnopoulous M (2007) Cancer-specific mutations in phosphatidylinositol 3-kinase. Trends Biochem Sci 32: 342–349

Walker EH, Perisic O, Ried C, Stephens L, Williams RL (1999) Structural insights into phosphoinositide 3-kinase catalysis and signalling. Nature 402: 313–320

Wullischegger S, Loewith R, Hall MN (2000) TOR signaling in growth and metabolism. Cell 124: 471–484

Yamashita A, Kashima I, Ohno S (2005) The role of SMG-1 in nonsense-mediated mRNA decay. Biochim Biophys Acta 1754: 305–315

You Z, Chahwan C, Bailis J, Hunter T, Russell P (2005) ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. Mol Cell Biol 25: 5363–5379

Yu J, Wjasow C, Backer JM (1998a) Regulation of the p85/p10alpha phosphatidylinositol 3'-kinase. Distinct roles for the n-terminal and c-terminal SH2 domains. J Biol Chem 273: 30199–30203

Yu J, Zhang Y, Mcllroy J, Rordorf-Nikolic T, Orr GA, Backer JM (1998b) Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic sub-unit by the p85 regulatory subunit. Mol Cell Biol 18: 1379–1387

Zhao JJ, Liu Z, Wang L, Shin E, Loda MF, Roberts TM (2005) The oncogenic properties of mutant p110alpha and p110beta phosphatidylinositol 3-kinases in human mammary epithelial cells. Proc Natl Acad Sci USA 102: 18443–18448

Zhou L, Vogt PK (2008) Class I PI3K in oncogenic cellular transformation. Oncogene 27: 5486–5496

Zou L, Elledge SJ (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300: 1542–1548

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