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Puzzle out the regulation mechanism of PI4KIIα activity

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Phosphatidylinositol 4-kinase IIα (PI4KIIα), the most abundant PI4K in mammalian cells, is best known for its essential role in providing the substrate for phosphatidylinositol 3-kinase (PI3K), an important kinase for phospholipid signaling. PI4KIIα also plays important roles in membrane trafficking, phagocytosis and the exo-endocytic cycle of synaptic vesicles. Its dysfunction results in tumor growth, spastic paraplegia and Gaucher's disease. Therefore, PI4KIIα may potentially be an important drug target.

The PI4K family comprises type II (55 kD PI4KIIα and PI4KIIβ) and type III PI4Ks (230 kD PI4KIIIα and 92 kD PI4KIIIβ). Phylogenetic analysis [1] based on their sequences (Figure 1A) reveals that PI4KIIIs are closed to PI3Ks thus belong to the PI 3/4-kinase family while PI4KIIs have more closed relationship with protein kinases (e.g., Actin fragmin kinase). This suggests that PI4KIIs should have a substrate binding pocket that is distinct from that of PI3Ks and PI4KIIIs. In addition, PI4KIIα contains a '-CCPCC-' motif that is palmitoylated *in vivo* and such palmitoylation is important for the kinase activity of PI4KIIα. Thus currently structural information available on PI3Ks and the well-developed specific PI3K inhibitors are not helpful for understanding the substrate binding specificity and the activity regulation of PI4KIIs.

Recently, the first crystal structure in the PI4K family, the catalytic domain of human PI4KIIα in an ADP-bound form was solved [2], and three novel insertions of PI4KIIα, namely I1 (a palmitoylation insertion), I2 (an RK-rich insertion) and I3 were found in this crystal structure (Figure 1B). These three insertions distinguish PI4KIIα from the structures of PI3Ks (Figure 1C). Furthermore, a distinct nucleotide-binding pocket of PI4KIIα differs notably from that of PI3Ks, which well explained the insensitivity of PI4KIIα to PI3Ks' inhibitors. Although many crystal structures of PIKs have already been solved, the precise nature of the substrate binding pocket is still not clear. The molecular dynamics (MD) simulation approach was utilized to identify a putative PI-binding pocket that was further evaluated using mutagenesis. More importantly, the MD simulation, biochemical and mutagenesis studies revealed a novel mechanism for the regulation of PI4KIIα's kinase activity, in which any perturbation of the interaction between PI4KIIα and the membrane will affect either the nucleotide binding or PI binding and subsequently modulate the kinase activity of PI4KIIα.

Current work [2] has provided insight into how the kinase activity of PI4KIIα is regulated at the molecular level. It also lays a foundation for designing specific PI4KIIα inhibitors and activators for future therapeutic applications. Importantly, it brings PI4KIIα out of the shadow of PI3K and sheds new light on the study of PI4KIIα in its own rite. Future studies could be focused on the following directions: (i) Discovery of specific inhibitors based on the ADP binding pocket of PI4KIIα. PI4KIIα has been proved as an oncoprotein and suppression of its expression level inhibits tumor growth [3]. Recently it was found that dual inhibition of EGFR at the protein and activity level via combinatorial blocking of PI4KIIα had a marked anti-tumor effect [4]. Thus PI4KIIα is undoubtedly an effective anti-tumor target. A subtype-specific inhibitor of PI4KIIα is important to avoid affecting activities of other PI4K/PI3K family members. (ii) Structural studies of the PI-bound form of PI4KIIα. Up to now most kinase inhibitors have been de-

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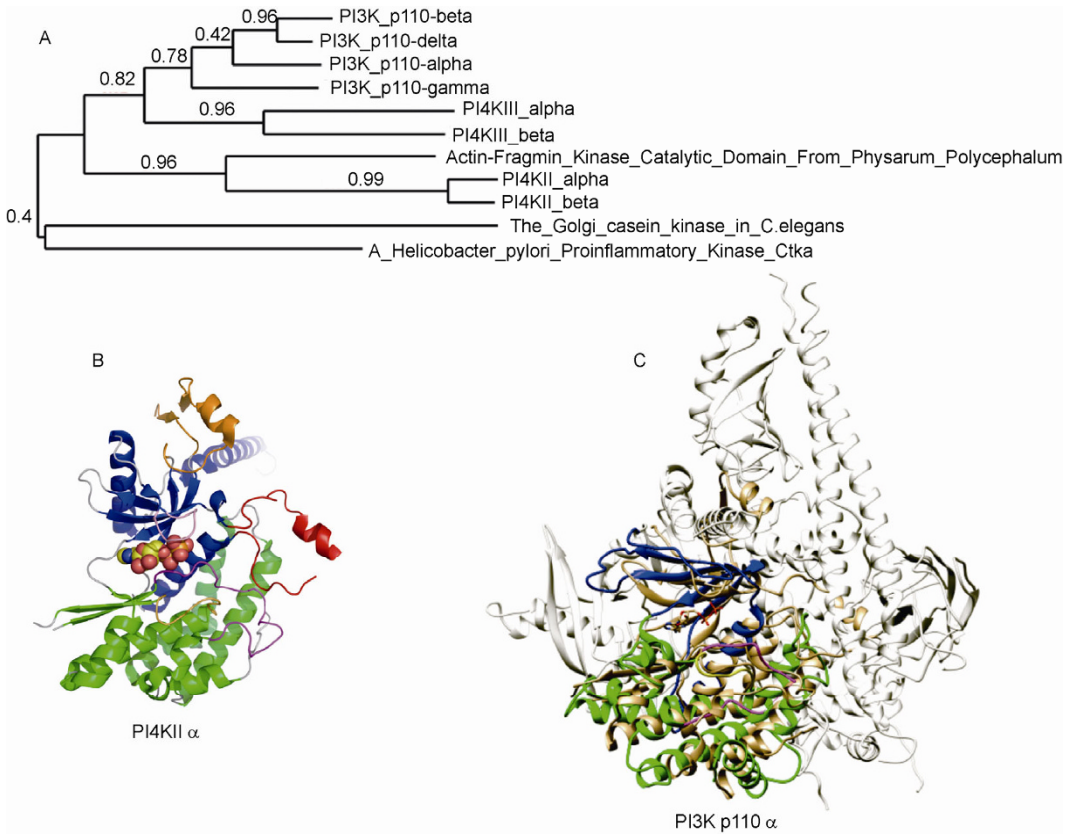

Figure 1 Structures of phosphatidylinositol kinases and phylogenetic analysis. A, Phylogenetic analysis of phosphatidylinositol kinases and protein kinases based on their amino acid sequences. The similarity of each pair of sequences is labeled accordingly. GenBank accession numbers for the amino acid sequences of these proteins are NP_060895 for human PI4KIIα, NP_060793 for human PI4KIIβ, NP_002641 for human PI4KIIIα, NP_002642.1 for human PI4KIII β , NP_006209 for human PI3Kα (p110α), AAB29081 for human PI3Kβ (p110β), NP_001269356 for human PI3Kγ (p110γ), NP_005017 for human PI3Kδ (p110δ), 1CJA_B for actin fragmin kinase, 4KQB_B for the Golgi casein kinase and 3AKK_D for a Helicobacter pylori proinflammatory kinase. The later three ones belong to protein kinases. The phylogenetic tree was produced by using the server "Phylogeny.fr" (http://www.phylogeny.fr) [1]. B, Crystal structure of the catalytic domain of human PI4KIIα in ADP bound form (PDB code 4HNE). The N-lobe and C-lobe are colored in blue and green, respectively. The palmitoylation insertion I1 and RK-rich insertion I2 are colored in red and gold, respectively. ADP is shown in a sphere model. C, Comparison of the structures of PI4KIIα and PI3Kα (PDB code 2RD0). The crystal structure of PI3Kα is shown as cartoon diagrams with the structure of PI4KIIα catalytic domain (colored in golden) superimposed. The views take the same angle in (B). The N- and C-lobes of the catalytic domain are colored in blue and green, respectively.

signed based on their ATP binding pocket [5]. There is no inhibitor available to block the binding of phospholipids due to lack of the phospholipid bound complex structures. However, considering that ATP is used widely by many kinds of proteins in various physiological pathways. The inhibitors to block the binding of ATP could not be easily specific and avoid side effects. Thus the ideal inhibitors of phospholipid kinases would be designed base on the phospholipid-binding pocket. While a putative PI-binding pocket of PI4KIIα has been discovered via molecular dynamics analysis [2], more experimental structural work (crystallography, NMR and EPR) should be performed to define precisely the interactions between PI4KIIα and PI, which is important to develop the specific inhibitors of blocking the binding of PI. (iii) Study compounds that can regulate the activity of PI4KIIα via tuning the fluctuation of membrane. Although the current work [2] has shown that cholesterol can regulate the kinase activity of PI4KIIα via non-direct interactions, more quantitative investigations are needed to precisely define how the activity of PI4KIIα is regulated via the changes of the membrane physical properties. It is also valuable to develop other compounds that can increase or decrease the physical fluctuation and fluidity of membrane and study how these new compounds affect the kinase activity of PI4KIIα. These compounds could be potentially useful for anti-tumor and the therapy of neurodegeneration diseases. (iv) Study the molecular mechanism of PI4KIIα palmitoylation. Although the enzyme for the palmitoylation of PI4KIIα has been proposed, its detailed molecular mechanism and how the palmitoylation of PI4KIIα is regulated are still awaiting to be solved.

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