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Phosphoinositide Spatially Free AKT/PKB Activation to all Membrane Compartments

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Abstract

Ser and Thr kinase AKT also known as protein kinase B (PKB) was discovered more than two and half decades ago and is one of the key downstream molecules in the phosphoinositide 3-kinase signaling pathways. The pleiotropic effects of this kinase have attracted intense interest and limelight in cancer biology, cancer therapy, diabetes, and cardiovascular diseases. Authors may refer to other more comprehensive and recent reviews on AKT/PKB (Manning and Cantley, 2007; Manning and Toker, 2017). AKT/PKB is one of the most enigmatic and most studied signaling molecule in cancers and is a significant therapeutic target (Brown and Banerji, 2017). Yet, how AKT/PKB activation couples with its downstream target/substrate molecules that function in diverse subcellular compartments remains obscure. Recent studies indicate the continuous interaction of AKT/PKB with PI3,4,5P₃ or PI3,4P₂ in a lipid membrane is required for its activation throughout the cells (Ebner et al., 2017). Here, we summarize the recent progress on the mechanism for phosphoinositide (PI3,4,5P₃ and PI3,4P₂) spatial control of AKT/PKB activation on the plasma membrane and endomembrane compartments.

Keywords

PI4,5P₂; PI3,4,5P₃; AKT/PKB; PI3K; Endosomes

INTRODUCTION

Activation of class I phosphatidylinositol 3-kinase (PI3K) downstream of different agonists or growth factor receptors induces the generation of the potent lipid messenger, phosphatidylinositol-3,4,5-trisphosphate (PI3,4,5P₃) that recruits and activates various downstream effector molecules. The Ser and Thr kinase AKT also known as protein kinase B (PKB) occupies the central position in phosphoinositide 3-kinase signaling. AKT/PKB

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Conflict of Interest Disclosure

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phosphorylation and regulation of downstream effector molecules such as GSK3 α/β , FOXO transcription factors, TOR, and the TSC complex plays a critical role in regulation of cell growth, cell survival, and cell metabolism (Jhanwar-Uniyal et al., 2017; Manning and Toker, 2017). The activation of AKT/PKB and its functions are intimately associated with the 3-phosphoinositides in lipid membranes, as well as the precursors of PI3,4,5P₃ that are required for its generation. The key portion of this focused review will summarize the recent progress made on how phosphoinositide molecules (PI3,4,5P₃ and PI3,4P₂) spatially confine AKT/PKB activation on plasma membrane and endomembrane compartments.

Phosphoinositide Signaling in Regulation of AKT/PKB Activation

Phosphoinositide is a minute but key component of the eukaryotic phospholipid bilayers in membrane and endomembrane (Tan et al., 2015; Thapa et al., 2016). There are eight phosphoinositides (e.g. PI, PI3P, PI4P, PI5P, PI3,4P₂, PI4,5P₂, PI3,5P₂, PI3,4,5P₃) regulating myriads of cellular functions (Hawkins and Stephens, 2016). Agonist stimulated activation of class I PI3K and PI3,4,5P₃ generation, and activation of downstream target molecules occupies the central position in phosphoinositide signaling (Vanhaesebroeck et al., 2012). Among the phosphoinositide isomers, PI3,4,5P₃ levels are low in quiescent cells but its level is induced several fold upon activation of class I PI3K downstream of various cell surfaces receptors, establishing PI3,4,5P₃ as a key lipid messenger in cell signaling (Choi et al., 2018; Fruman et al., 2017). AKT/PKB gained prominence in cell signaling when its activation was discovered downstream of class I PI3K and PI3,4,5P₃ generation. Now, PI3K/AKT signaling is one of the most commonly targeted signaling pathways in therapeutic treatment of cancers (LoRusso, 2016; Ricciardi et al., 2017). There are two inhibitors of the PI3K/AKT signaling pathway, namely Idelalisib and Copansilib, that entered the clinic in 2014 and 2017 for therapeutic treatments of chronic lymphocytic leukemia and follicular lymphoma, respectively.

AKT/PKB is Serine/Threonine kinase with three isoforms, AKT1-AKT3, each encoded by separate genes (Manning and Toker, 2017). AKT1 is ubiquitous whereas AKT2 and AKT3 are major isoforms in insulin responsive tissues and the brain, respectively. The domain architecture of the AKT isoforms includes an N-terminal PH domain, an unstructured linker sequence, a conserved kinase domain, and a flexible C-terminal regulatory domain (Chu et al., 2018) (Figure 1A). In unstimulated conditions, the tight interaction between the PH and kinase domain through hydrophobic interactions keep AKT/PKB in a closed and inactive conformation that is largely in the cytosol. In this conformation, the PH domain and activation loop in the kinase domain are restricted from interacting with PI3,4,5P₃ and protein substrates, respectively. The generation of the PI3,4,5P₃ and/or PI3,4P₂ phosphoinositide messengers in a membrane provide specific docking sites for the PH domain, recruiting AKT/PKB and inducing a conformational change leading to activation (Figure 1B). Phosphoinositide binding and conformation changes in AKT/PKB further provide the access to PDK1 that phosphorylates its Threonine residue (T308 in AKT1) in the kinase domain, followed by phosphorylation of its Serine residue (S473 in AKT1) in the hydrophobic motif in the C-tail by the mTORC2 complex (Manning and Toker, 2017). The phosphorylation of T308 leads to activation loop ordering and high-affinity substrate binding, while phosphorylation on S473 in the hydrophobic motif orders the C helix for

efficient catalysis (Lucic et al., 2018). PI3,4,5P₃ also provides the docking site for the PH domain of PDK1, thus simultaneously bringing PDK1 and AKT/PKB together at the membrane. The phosphorylation of both T308 and S473 residues is required for maximal activation of AKT/PKB. Rapid conversion of PI3,4,5P₃ back to PI4,5P₂ by 3-phosphatase PTEN terminates the PI3,4,5P₃ signal for AKT/PKB conformational change and activation, thus bringing it back to the closed conformation. However, PI3,4,5P₃ conversion to PI3,4P₂ by 5-phosphatases (e.g. SHIP1, SHIP2, SYNJ2, INPP5B, INPP5E and INPP5J) continues to activate AKT/PKB as the PH domain of AKT/PKB binds indiscriminately to PI3,4P₂ vs PI3,4,5P₃. Dephosphorylation of PI3,4P₂ by 4-phosphatases (e.g. INPP4A and INPP4B) to PI3P on endosomal membranes will terminate the activation of AKT/PKB (Li Chew et al., 2015). Similarly, protein phosphatases PP2A and PHLPP dephosphorylate T308 and S473 residues of AKT1, terminating the activation of AKT/PKB (Manning and Toker, 2017).

Although the spatial generation of PI3,4,5P₃ and subsequent AKT/PKB activation at the plasma membrane prevails in the phosphoinositide signaling field, there are recent studies that have defined the indispensable role of continued interaction of AKT/PKB with PI3,4,5P₃ or PI3,4P₂ in a membrane for its activation and functions throughout cellular compartments (Ebner et al., 2017). As receptors that activate PI 3-kinase and AKT/PKB are rapidly endocytosed after agonist activation, a precise understanding of the spatial AKT/PKB activation is key for effective targeting of AKT/PKB in combination with Class I PI3Ks inhibitors (LoRusso, 2016).

Downstream Effector Molecules of Activated AKT/PKB

AKT/PKB is a broad-spectrum Ser/Thr kinase that phosphorylates over 100 proteins. Discovery of GSK3 α /GSK3 β as an AKT/PKB substrate, and identification of minimal consensus AKT/PKB phosphorylation motif (R-X-R-X-XS/T-Q) led to subsequent recognition of many target proteins of active AKT/PKB (Hermida et al., 2017; Manning and Toker, 2017). These proteins include protein kinases, lipid kinases, transcription factors, small G protein regulators, metabolic enzymes, E3 ubiquitin ligases, cell cycle regulators, and regulators of vesicle trafficking, all functioning at different subcellular compartments. These define the AKT/PKB regulation of different cellular functions including cell growth, cell survival, cell differentiation, and metabolism (Manning and Toker, 2017). Among AKT/PKB substrates or effector molecules, the FOXO family of transcriptional factors, GSK3 α /GSK3 β , and the mTORC1 inhibitor TSC complex are the most extensively studied downstream effector molecules of activated AKT/PKB.

The AKT/PKB activation involves sequential phosphorylation of PI to PI4P and PI4,5P₂, which is the substrate of agonist stimulated class I PI3K, resulting in the PI3,4,5P₃ that recruits AKT/PKB to membranes from the cytosol which gets phosphorylated by PDK1 and the mTORC2 complex. Active AKT/PKB has many substrates that function at subcellular compartments separate from the plasma membrane, such as the FOXO family of transcription factors that go into the nucleus, the TSC complex at lysosomes, pro-apoptotic proteins Bax and Bad at the interface of mitochondria, and MDM2 in the cytosol (Manning and Toker, 2017; Stiles, 2009). This led to a widely accepted belief that active AKT/PKB locked in active conformation detaches from plasma membrane, uncoupling it from its

activating stimuli and spatial control by PI3,4,5P₃ or PI3,4P₂, and diffuses into cytosol where it encounters its substrate molecules. It is important to note that high-micromolar affinity of inactive AKT/PKB with its substrates suggests that cytosolic AKT/PKB in its inactivate state is unlikely to bind and phosphorylate its substrates. Further, recent studies indicate an absolute requirement of binding PI3,4,5P₃ or PI3,4P₂ phosphoinositide in a membrane for optimal AKT/PKB activation (Ebner et al., 2017), which raises discrepancies in spatial activation of AKT/PKB and its functions via its substrate molecules. Is AKT/PKB phosphorylation of its substrate molecules restricted only to a membrane containing PI3,4,5P₃ or PI3,4P₂? If this is the case, how can AKT/PKB be activated on endosomal membranes that lack the precursor lipids for PI3,4,5P₃ generation?

AKT/PKB Activation is Restricted to PI3,4,5P₃ or PI3,4P₂ Containing Membrane

Transient character of the PI3,4,5P₃-AKT/PKB interaction and recruitment of activated AKT/PKB to the plasma membrane led to the prevailing model of AKT/PKB activation at plasma membrane followed by dissociation of activated AKT/PKB from the plasma membrane and diffusion to cytosol where it phosphorylates its substrate molecules (Manning and Toker, 2017). Yet, AKT/PKB upon dissociation from PI3,4,5P₃ or PI3,4P₂ is robustly autoinhibited due to an intramolecular inhibitory interaction between its PH domain and kinase domain and undergoes rapid dephosphorylation. This data indicates that active AKT/PKB that is competent for substrate phosphorylation is predominantly associated with a PI3,4,5P₃/PI3,4P₂ containing membrane surface (Ebner et al., 2017; Lucic et al., 2018). This is consistent as PI3,4,5P₃ or PI3,4P₂ binding to the PH domain allosterically activates AKT/PKB and confines active AKT/PKB to a membrane where substrate binding and phosphorylation would occur (Ebner et al., 2017). Upon attenuation of PI3,4,5P₃ or PI3,4P₂ signal, AKT/PKB dissociates from membrane leading to conformational changes and exposure of its phosphorylated activation loop and hydrophobic motif to PHLPP and PP2A phosphatases, respectively. However, a study using a semisynthetic, site-specifically modified phospho-form of AKT/PKB protein revealed an additional mechanism for AKT/PKB activation in which dual phosphorylation of C-tail Ser 477 and Thr 479 by Cdk2/cyclinA2 reduces the PH domain autoinhibition in AKT/PKB, and can function independent of a membrane interaction (Chu et al., 2018). Yet, this pathway for AKT/PKB activation may be distinct from the agonist stimulated pathways. Similarly, methylation of the AKT/PKB linker region by histone methyltransferase SETDB1 induced by PI3,4,5P₃ interaction with PH domain of AKT/PKB is known to sustain the active form of AKT/PKB (Guo et al., 2019), but it is not clear that this modification changes a requirement for the continuous interaction of AKT/PKB with PI3,4,5P₃/PI3,4P₂ membrane for its activity.

The major anionic phospholipid in the inner leaflet of plasma membrane, phosphatidylserine also cooperates with PI3,4,5P₃ and induction of the conformational change in AKT/PKB, promoting its phosphorylation in T308 and S473 residues (Huang et al., 2011). Phosphatidylserine binds to the basic residues R15 and K20 adjacent to the PI3,4,5P₃-binding pocket in the PH domain, and PI3,4,5P₃ alone is much less effective in inducing the conformational change and AKT/PKB activation. This further supports a membrane requirement and is reminiscent of other enzymes that are activated by lipid messengers.

Endosomal Phosphoinositide in Regulation of AKT/PKB Activation

Current dogma indicates that the PI3,4,5P₃ lipid messenger generated at the plasma membrane upon activation of growth factor receptors and class I PI3Ks is primarily responsible for AKT/PKB activation. Conversely, phosphoinositide in the endomembrane compartments is emerging as crucial site for AKT/PKB activation (Jethwa et al., 2015; Naguib, 2016; Sato et al., 2003). Discrepancies brought by the requirement of PI3,4,5P₃ and/or PI3,4P₂ lipid membranes for AKT/PKB activation and its confinement at plasma membrane when its substrates are at different subcellular compartments can be addressed by the concept that AKT/PKB can be active on endomembrane compartments. This provides a mechanism for AKT/PKB control of target molecules after the activated receptor is endocytosed or activated in endomembrane compartments. Potentially, this could extend to multiple subcellular compartments where AKT/PKB substrates are spatially localized and function (e.g. endosomal trafficking vesicles, lysosomes, Golgi apparatus, and nuclear membrane and non-membrane nuclear sites). Given the much higher surface area occupied by the endomembrane system throughout cells compared to the plasma membrane, endomembrane PI3,4,5P₃ or/and PI3,4P₂ are positioned to activate AKT/PKB at subcellular compartments distal to the plasma membrane and closer to key substrates (Figure 1C).

Although PI3,4,5P₃ and PI3,4P₂ indiscriminately activate AKT/PKB, PI3,4,5P₃ is the primary stimuli at plasma membrane as PI3,4P₂ generation lags that of PI3,4,5P₃ in many cases (Hawkins and Stephens, 2016; Jethwa et al., 2015). Inositol phosphatases SHIP1/SHIP2 dephosphorylate PI3,4,5P₃ to generate PI3,4P₂ in plasma membrane and endosomes (Goulden et al., 2019). PI3,4,5P₃ is viewed as a short-lived lipid messenger at plasma membrane unlike PI3,4P₂ that operates at both plasma membrane and endosomes, highlighting the critical role of the PI3,4P₂ lipid messenger in AKT/PKB activation both in plasma membrane and endomembrane. PI3,4P₂ selectively activates AKT2 at both plasma membrane and early endosomes, whereas PI3,4,5P₃ selectively stimulates AKT1 and AKT3 at plasma membrane (Liu et al., 2018). PDK1 also indiscriminately binds PI3,4,5P₃ and PI3,4P₂, and mTORC2 largely functions in the endosomal/ lysosomal system (Boulbes et al., 2011). These combined data support the existence of cellular machinery to activate AKT/PKB in endomembrane compartments. But how is the endosomal compartment equipped to generate PI3,4,5P₃ or PI3,4P₂ that activates AKT/PKB?

The activation of class I PI3K and generation of PI3,4,5P₃ lipid messenger in plasma membrane and/or in endomembrane is intimately associated with different receptors. Many receptors including EGFR, insulin receptor, PDGFR, and GPCR continue to signal at endosomal compartments, providing the molecular clue for activation of class I PI3K and PI3,4,5P₃ generation at endosomes (Murphy et al., 2009). Yet, the endosomal compartment lacks detectable amounts of the phosphoinositide substrates required for PI3,4,5P₃ generation. These issues could be solved by scaffolding of all the PI 3-kinase signaling pathway components into a complex. The IQGAP1 scaffolding molecule orchestrates the agonist stimulated assembly of all the phosphoinositide kinases required to synthesize PI3,4,5P₃ from PI. This streamlines PI3,4,5P₃ synthesis, and as PDK1 and AKT/PKB are also within this scaffold, activation downstream of receptors have the potential to occur at any subcellular membrane compartment that contains PI, as well as the plasma membrane

(Choi et al., 2016) (Figure 2). In the IQGAP1 complex the PI3,4,5P₃ can be dephosphorylated by tumor suppressor PTEN which is recruited to the complex by interactions with the p85 subunit of PI3K (Chagpar et al., 2010), potentially to IQGAP1 itself (Choi et al., 2016), or via direct binding to PI3P or other lipid moieties in endosomal surface (Malek et al., 2017; Naguib et al., 2015).

In addition to dephosphorylation of PI3,4,5P₃ to PI3,4P₂, different isoforms of class II PI3K contribute to synthesis of different pools of endosomal PI3,4P₂ (Gulluni et al., 2019). For example, PI3KC2 γ generated PI3,4P₂ in endosomes controls the AKT2 activation downstream of insulin signaling (Braccini et al., 2015); PI3KC2 β generates PI3,4P₂ at late endosomes/lysosomes (Marat et al., 2017); PI3KC2 α plays a major role in PI3,4P₂ synthesis at clathrin-coated structures (Posor et al., 2013). With PI4P being the most abundant phosphoinositide species, the activity of class II PI3K may generate the requisite amount of PI3,4P₂ for AKT/PKB activation in endosomal system.

The Scaffolding of the PI3K Pathway Components and AKT Changes Everything

The activation mechanisms for AKT/PKB are based upon studies of an enzyme that is free of constraints other than PI3,4,5P₃, PI3,4P₂, and membrane binding (Risso et al., 2015). The incorporation of AKT/PKB into a scaffold with other PI 3-kinase pathway kinases must intrinsically change our thinking of how AKT/PKB is activated and interacts with its substrates. The concept that PI3,4,5P₃/PI3,4P₂ and PDK1 are required seems clear, but the roles of interaction with IQGAP1 or associated proteins clearly could impact how AKT/PKB activity is controlled, and how AKT/PKB interacts with its substrates (Figure 2) (Choi et al., 2016). The impact of IQGAP1 and potentially other scaffolding molecules on AKT/PKB activation complicates the dogma and indicates that AKT/PKB activity may be much more spatially controlled than previously appreciated.

Conclusion

Although phosphoinositide signaling and AKT/PKB represent one of the most extensively studied signaling pathways and its downstream effector molecule, respectively, many unresolved issues remain. AKT/PKB activation and its substrate phosphorylation on phosphoinositide endomembrane needs to be defined more precisely by focusing more on compartmentalized and site-specific AKT/PKB activation and substrate phosphorylation. As only handful of substrate molecules are localized in endomembrane, how is AKT/PKB activation coupled to AKT/PKB phosphorylation of those substrate molecules beyond plasma membrane and endomembrane? In addition to class I PI3K, the precise role of class II PI3K in generation of endosomal PI3,4,5P₃ and PI3,4P₂, and the possible role of scaffolding molecules in AKT/PKB activation needs to be defined. Similarly, how somatic mutations in AKT/PKB in different cancers affect activation and phosphoinositide membrane dependency needs to be defined.

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REFERENCES

- Boulbes DR, Shaiken T, and Sarbassov dos D (2011). Endoplasmic reticulum is a main localization site of mTORC2. *Biochem Biophys Res Commun* 413, 46–52. [PubMed: 21867682]
- Braccini L, Ciraolo E, Campa CC, Perino A, Longo DL, Tibolla G, Pregnolato M, Cao Y, Tassone B, Damilano F, et al. (2015). PI3K-C2gamma is a Rab5 effector selectively controlling endosomal Akt2 activation downstream of insulin signalling. *Nat Commun* 6, 7400. [PubMed: 26100075]
- Brown JS, and Banerji U (2017). Maximising the potential of AKT inhibitors as anti-cancer treatments. *Pharmacol Ther* 172, 101–115. [PubMed: 27919797]
- Chagpar RB, Links PH, Pastor MC, Furber LA, Hawrysh AD, Chamberlain MD, and Anderson DH (2010). Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* 107, 5471–5476. [PubMed: 20212113]
- Choi S, Hedman AC, Sayedyahosseini S, Thapa N, Sacks DB, and Anderson RA (2016). Agonist-stimulated phosphatidylinositol-3,4,5-trisphosphate generation by scaffolded phosphoinositide kinases. *Nat Cell Biol* 18, 1324–1335. [PubMed: 27870828]
- Choi S, Houdek X, and Anderson RA (2018). Phosphoinositide 3-kinase pathways and autophagy require phosphatidylinositol phosphate kinases. *Adv Biol Regul* 68, 31–38. [PubMed: 29472147]
- Chu N, Salguero AL, Liu AZ, Chen Z, Dempsey DR, Ficarro SB, Alexander WM, Marto JA, Li Y, Amzel LM, et al. (2018). Akt Kinase Activation Mechanisms Revealed Using Protein Semisynthesis. *Cell* 174, 897–907 e814. [PubMed: 30078705]
- Ebner M, Lucic I, Leonard TA, and Yudushkin I (2017). PI(3,4,5)P3 Engagement Restricts Akt Activity to Cellular Membranes. *Mol Cell* 65, 416–431 e416. [PubMed: 28157504]
- Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, and Abraham RT (2017). The PI3K Pathway in Human Disease. *Cell* 170, 605–635. [PubMed: 28802037]
- Goulden BD, Pacheco J, Dull A, Zewe JP, Deiters A, and Hammond GRV (2019). A high-avidity biosensor reveals plasma membrane PI(3,4)P2 is predominantly a class I PI3K signaling product. *J Cell Biol* 218, 1066–1079. [PubMed: 30591513]
- Gulluni F, De Santis MC, Margaria JP, Martini M, and Hirsch E (2019). Class II PI3K Functions in Cell Biology and Disease. *Trends Cell Biol*.
- Guo J, Dai X, Laurent B, Zheng N, Gan W, Zhang J, Guo A, Yuan M, Liu P, Asara JM, et al. (2019). AKT methylation by SETDB1 promotes AKT kinase activity and oncogenic functions. *Nat Cell Biol* 21, 226–237. [PubMed: 30692625]
- Hawkins PT, and Stephens LR (2016). Emerging evidence of signalling roles for PI(3,4)P2 in Class I and II PI3K-regulated pathways. *Biochem Soc Trans* 44, 307–314. [PubMed: 26862220]
- Hermida MA, Dinesh Kumar J, and Leslie NR (2017). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Adv Biol Regul* 65, 5–15. [PubMed: 28712664]
- Huang BX, Akbar M, Kevala K, and Kim HY (2011). Phosphatidylserine is a critical modulator for Akt activation. *J Cell Biol* 192, 979–992. [PubMed: 21402788]
- Jethwa N, Chung GH, Lete MG, Alonso A, Byrne RD, Calleja V, and Larijani B (2015). Endomembrane PtdIns(3,4,5)P3 activates the PI3K-Akt pathway. *J Cell Sci* 128, 3456–3465. [PubMed: 26240177]
- Jhanwar-Uniyal M, Amin AG, Cooper JB, Das K, Schmidt MH, and Murali R (2017). Discrete signaling mechanisms of mTORC1 and mTORC2: Connected yet apart in cellular and molecular aspects. *Adv Biol Regul* 64, 39–48. [PubMed: 28189457]
- Li Chew C, Lunardi A, Gulluni F, Ruan DT, Chen M, Salmena L, Nishino M, Papa A, Ng C, Fung J, et al. (2015). In Vivo Role of INPP4B in Tumor and Metastasis Suppression through Regulation of PI3K-AKT Signaling at Endosomes. *Cancer Discov* 5, 740–751. [PubMed: 25883022]
- Liu SL, Wang ZG, Hu Y, Xin Y, Singaram I, Gorai S, Zhou X, Shim Y, Min JH, Gong LW, et al. (2018). Quantitative Lipid Imaging Reveals a New Signaling Function of Phosphatidylinositol-3,4-Bisphosphate: Isoform-and Site-Specific Activation of Akt. *Mol Cell* 71, 1092–1104 e1095. [PubMed: 30174291]
- LoRusso PM (2016). Inhibition of the PI3K/AKT/mTOR Pathway in Solid Tumors. *J Clin Oncol* 34, 3803–3815. [PubMed: 27621407]

- Lucic I, Rathinaswamy MK, Truebestein L, Hamelin DJ, Burke JE, and Leonard TA (2018). Conformational sampling of membranes by Akt controls its activation and inactivation. *Proc Natl Acad Sci U S A* 115, E3940–E3949. [PubMed: 29632185]
- Malek M, Kielkowska A, Chessa T, Anderson KE, Barneda D, Pir P, Nakanishi H, Eguchi S, Koizumi A, Sasaki J, et al. (2017). PTEN Regulates PI(3,4)P₂ Signaling Downstream of Class I PI3K. *Mol Cell* 68, 566–580 e510. [PubMed: 29056325]
- Manning BD, and Cantley LC (2007). AKT/PKB signaling: navigating downstream. *Cell* 129, 1261–1274. [PubMed: 17604717]
- Manning BD, and Toker A (2017). AKT/PKB Signaling: Navigating the Network. *Cell* 169, 381–405. [PubMed: 28431241]
- Marat AL, Wallroth A, Lo WT, Muller R, Norata GD, Falasca M, Schultz C, and Haucke V (2017). mTORC1 activity repression by late endosomal phosphatidylinositol 3,4-bisphosphate. *Science* 356, 968–972. [PubMed: 28572395]
- Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, and Bunnett NW (2009). Endosomes: a legitimate platform for the signaling train. *Proc Natl Acad Sci U S A* 106, 17615–17622. [PubMed: 19822761]
- Naguib A (2016). Following the trail of lipids: Signals initiated by PI3K function at multiple cellular membranes. *Sci Signal* 9, re4. [PubMed: 27188443]
- Naguib A, Bencze G, Cho H, Zheng W, Tocilj A, Elkayam E, Faehnle CR, Jaber N, Pratt CP, Chen M, et al. (2015). PTEN functions by recruitment to cytoplasmic vesicles. *Mol Cell* 58, 255–268. [PubMed: 25866245]
- Posor Y, Eichhorn-Gruenig M, Puchkov D, Schoneberg J, Ullrich A, Lampe A, Muller R, Zarbakhsh S, Gulluni F, Hirsch E, et al. (2013). Spatiotemporal control of endocytosis by phosphatidylinositol-3,4- bisphosphate. *Nature* 499, 233–237. [PubMed: 23823722]
- Ricciardi MR, Mirabilii S, Licchetta R, Piedimonte M, and Tafuri A (2017). Targeting the Akt, GSK-3, Bcl-2 axis in acute myeloid leukemia. *Adv Biol Regul* 65, 36–58. [PubMed: 28549531]
- Risso G, Blaustein M, Pozzi B, Mammi P, and Srebrow A (2015). Akt/PKB: one kinase, many modifications. *Biochem J* 468, 203–214. [PubMed: 25997832]
- Sato M, Ueda Y, Takagi T, and Umezawa Y (2003). Production of PtdInsP₃ at endomembranes is triggered by receptor endocytosis. *Nat Cell Biol* 5, 1016–1022. [PubMed: 14528311]
- Stiles BL (2009). PI-3-K and AKT: Onto the mitochondria. *Adv Drug Deliv Rev* 61, 1276–1282. [PubMed: 19720099]
- Tan X, Thapa N, Choi S, and Anderson RA (2015). Emerging roles of PtdIns(4,5)P₂--beyond the plasma membrane. *J Cell Sci* 128, 4047–4056. [PubMed: 26574506]
- Thapa N, Tan X, Choi S, Lambert PF, Rapraeger AC, and Anderson RA (2016). The Hidden Conundrum of Phosphoinositide Signaling in Cancer. *Trends Cancer* 2, 378–390. [PubMed: 27819060]
- Vanhaesebroeck B, Stephens L, and Hawkins P (2012). PI3K signalling: the path to discovery and understanding. *Nat Rev Mol Cell Biol* 13, 195–203. [PubMed: 22358332]

Figure 1. AKT/PKB Activation Spatially Confined at Phosphoinositide Membrane

A, Schematic diagram of AKT/PKB depicting its domain architecture which includes an N-terminal PH domain, an unstructured linker domain, and a conserved kinase domain followed by flexible C-terminal regulatory domain (C-tail).

B, Phosphoinositide binding to PH domain activates AKT/PKB. The intramolecular interaction between the PH domain and the kinase domain masks the activation loop in the kinase domain. The engagement of its PH domain with membrane phosphoinositide (PI3,4,5P₃ or PI3,4P₂) induces the conformational changes in AKT/PKB and its phosphorylation by PDK1 (at T308 in kinase domain) followed by mTORC2 complex

phosphorylation (at S473 in C-tail). Phosphatidylserine, the most predominant anionic phospholipid also binds to PH domain of AKT/PKB and collaborates with PI3,4,5P₃ in inducing activation of AKT/PKB.

C, Endosomal phosphoinositide plays critical role in activation of AKT/PKB at various subcellular compartments. PI3,4,5P₃ and PI3,4P₂ generation at plasma membrane upon agonist activated class I PI3K is the initial stimuli for AKT/PKB activation at plasma membrane. The conversion of PI3,4,5P₃ into PI3,4P₂ at plasma membrane or endomembrane or generation of PI3,4,5P₃ and PI3,4P₂ at endomembrane system engages PH domain of AKT/PKB sustaining its activation and its phosphorylation of substrate molecules at different subcellular compartments. RTK, receptor tyrosine kinase; GPCR, G-protein coupled receptor; Int., integrins; PM, plasma membrane.

Figure 2. IQGAP1 Scaffolding of PI3K Pathway Components at Plasma Membrane and Endomembrane

Multi-scaffolding molecule IQGAP1 assembles PI4K, PIP5K1α and PI3K at functional proximity for streamlining PI3,4,5P₃ synthesis from PI and AKT/PKB activation. IQGAP1 scaffolding of PI3K pathway components has potential to occur at any cellular membrane including plasma membrane and endomembrane compartments. This introduces novel concept and mechanism in activation of AKT/PKB. RTK, receptor tyrosine kinase; GPCR, G-protein coupled receptor; Int., integrins; PM, plasma membrane.