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Review article

Role of phosphatidylinositol-4,5-bisphosphate 3-kinase signaling in vesicular trafficking

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ABSTRACT

Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3Ks) are regulatory enzymes involved in the generation of lipid species that modulate cellular signaling pathways through downstream effectors to influence a variety of cellular functions. Years of intensive study of PI3Ks have produced a significant body of literature in many areas, including that PI3K can mediate intracellular vesicular trafficking and through these actions contribute to a number of important physiological functions. This review focuses on the crucial roles that PI3K and AKT, a major downstream partner of PI3K, play in the regulation of vesicle trafficking during various forms of vesicular endocytosis and exocytosis.

Contents

1. Introduction

Phosphoinositides (PIs) are ubiquitous regulatory molecules present in the plasma membrane of many cell types. PIs are involved in orchestrating several signaling events in eukaryotic cells and in modulating the temporal and spatial specificity of intracellular signaling pathways. In contrast to other phospholipids, the head group of PIs can undergo reversible phosphorylation to generate multiple PI species that participate in various signaling cascades which regulate key cellular functions. PI synthesis is mediated by the action of phosphatidylinositol kinases that phosphorylate different hydroxyl groups on the phosphatidylinositol (PtdIns) backbone. Early studies defined a canonical PI signaling pathway that involved sequential phosphorylation of PtdIns by PtdIns 4-kinase and PtdIns-4-P 5-kinase to generate phosphatidylinositol 4,5-biphosphate (PIP2), which is the major target for phospholipase C (PLC) mediated hydrolysis [1,2]. Continued study during the following decades expanded our understanding of the levels of complexity associated with this signaling pathway by revealing the importance of PI kinases.

The most heavily studied members of the phosphatidylinositol kinase family are the phosphoinositide 3-kinases (PI3Ks) that catalyze

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phosphorylation of the 3′ inositol ring to generate phosphatidylinositol-3 phosphate (PI3P) [2,3]. Members of the PI3-kinase family have been implicated in many cellular processes, including controlling cellular growth and survival, regulating cytoskeletal remodeling through actin reorganization and modulation of intracellular vesicular trafficking that broadly affect the endocytic and exocytic processes [2,4,5]. Here we will focus on the extensive research conducted on the regulatory role of PI3-kinase signaling in vesicular endocytosis and exocytosis.

2. Structure of PI3-kinase family members

Since its discovery in immunoprecipitates of $pp60^{v\text{-}src}$ and $T/pp^{60c\text{-}src}$ from transformed cells, isoforms of the PI3K family have been isolated from various organisms ranging from yeast to human [6,7]. Based on structural arrangements and substrate specificity, PI3Ks have been classified into four major classes, IA, IB, II and III. Each member of the PI3K family harbors a catalytic domain and a C2 domain that have been shown to interact with phospholipids and to recruit and tether the molecule to the plasma membrane [8–10]. Mammalian PI3K was shown to be activated downstream of protein tyrosine kinase signaling [11,12]. Structurally, PI3K is composed of two separate subunits, an 85 kDa regulatory subunit known as p85 that displays two specific isoforms, p85α and p85β; and a 110 kDa catalytic subunit known as p110 with p110α, p110β and p110δ subtypes. Analysis of the structure of the protein indicates that the regulatory subunit is comprised of 724 amino acids and harbors an N-terminal src-homology 3 domain (SH3) and two src-homology 2 domains (SH2) that are interconnected by a coiled coil region known as the inter-SH2-domain [13,14]. The SH domains of the p85 regulatory subunit are involved in mediating protein-protein interactions and in converting the enzyme to its active state. The SH2 domains interact specifically with the phosphotyrosine motif of scaffolding proteins to activate and recruit the catalytic subunit to the plasma membrane. The SH3 domain binds to molecular partners enriched in short proline-rich stretches of amino acid residues [5,15–17].

Cloning of the PI3K catalytic subunit by protein microsequencing established that p110 is the epicenter of PI3K activity, functionally organized in a heterodimeric p85–p110 complex [18,19]. Mammalian p110α shows high homology to the *Saccharomyces cerevisiae* VPS34 protein which is involved in targeted endosomal sorting of proteins in yeast vacuoles and also plays a critical role in vacuolar morphogenesis during the process of yeast budding [20,21]. Several lines of experimental evidence indicate that VPS34 harbors PI3-kinase activity, including findings that mutation of conserved residues in p110 disrupt kinase enzymatic activity [22,23]. This kinase activity can phosphorylate an array of lipid moieties that target a number of downstream factors thus regulating many aspects of cellular function.

3. AKT and other downstream effectors of PI3K

With the advent of bioinformatics screening, various conserved protein domain structures in signaling molecules were identified. Plekstrin homology (PH) domains emerged as one of the common conserved domains in PI3K downstream effectors [24,25]. AKT (or protein kinase B), a central effector of the PH domain containing PI3K dependent pathway, shares homology with the protein kinase A (PKA) family of serine/threonine kinases and with the retroviral transforming protein v-AKT [26,27]. AKT is recognized as a direct effector of the PI3K signaling cascade which is activated in response to various growth factors and cytokines [28,29]. The highly conserved PI3K/AKT pathway becomes activated in a multistep manner involving ligand-receptor mediated activation of PI3K which triggers the conversion of membrane bound PtdIns [3,4] P_2 (PIP_2) to PtdIns [3,4,5] P_3 (PIP_3), thereby providing an AKT docking site for subsequent phosphorylation (Thr308) and partial activation by PDK1 [30,31]. In addition to phosphorylation at Thr308, AKT activation also requires phosphorylation at a different site on the regulatory domain (Ser473), preferentially via mTORC2 or in a DNA-dependent protein kinase (DNA-PK) dependent fashion to produce maximal activity [32,33]. Interestingly, some studies reported a PI3K independent mechanism of AKT activation via G-protein coupled receptor signaling, Ca^{2+}-calmodulin-dependent kinases or with members of the IκB kinase family [30,34–38].

Genetic manipulation of AKT to generate knockout and knockdown mouse models and *in vitro* mutation studies has provided substantial insight into its role in mediating important physiological functions. A number of studies implicate AKT as a primary regulator of cell growth and survival through targeting members of the pro-apoptotic pathway such as Bcl-2 related protein and Bcl-2-associated death promoter (BAD) [39,40]. AKT mediated phosphorylation of the BAD protein abolishes its pro-apoptotic functions thereby facilitating cell survival [41–43]. The growth promoting effect of AKT is mediated by positively modulating the cell cycle process. AKT activation via growth factors promotes c-myc transcription and accelerates cell cycle progression through enhanced expression of D cyclins, and minimizes negative regulators of the cell cycle process such as p21Cip1 and p27Kip1 resulting in a faster exit from the G0 phase [44,45]. Genetic studies provide strong evidence that the PI3K/AKT pathway mediates muscle hypertrophy in both skeletal and cardiac muscle [46,47]. The PI3K/AKT pathway also plays a critical role in regulating signaling events downstream of insulin signaling. Insulin receptor (IR) activation leads to AKT binding to lipid moieties (PIP3) and subsequent activation of exocytic vesicle trafficking and glucose uptake in response to insulin [48,49].

Although AKT acts as a central player in the downstream effects of PI3K signaling, the PI3K axis regulates a broader set of signaling pathways to mediate diverse cellular functions. The initiation of cellular mitogenesis entails complex signaling processes that activate growth factor receptors to initiate a variety of intracellular events leading to cell proliferation. The PI3K pathway was shown to be the common target of receptor tyrosine kinases [50]. Activation of the PI3K cascade by the oncoprotein RAS and its association with numerous growth factor receptors demonstrates the important role of the signaling axis in cell growth and proliferation [11,51]. These mitogenic effects of PI3K appear to occur in a cell-type and stimulus dependent fashion. Studies clearly demonstrate that suppressing PI3K activity through inhibitory peptides abolishes platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) triggered DNA synthesis. However, suppression of PI3K activity shows no effect on suppressing DNA synthesis in response to colony stimulating factor-1 (CSF-1), bombesin or lysophosphatidic acid [2,52]. PI3K has been shown to induce EGF-mediated mitogenic signaling in mesenchymal cells. Blocking PI3K activity through various pharmacological and molecular inhibitors causes significant inhibition of EGF-induced DNA synthesis [53,54].

PI3K signaling triggers multiple pathways that converge to play a role in the apoptotic process. Once the catalytic p110 subunit is activated by interaction with the p85 regulatory subunit, p110 activates two distinct phosphatidylinositol-dependent kinases known as PDK1 and PDK2. The PDKs are serine/threonine protein kinases that phosphorylate and activate AKT. AKT forms the downstream effector of PI3K signaling that mediates its anti-apoptotic function at the mitochondria level via AKT mediated phosphorylation and activation of BAD proteins [41,55]. Additionally, Iκ-kinase phosphorylation via the AKT-dependent pathway leads to subsequent degradation of I-κB and subsequent NF-κB nuclear translocation leading to activation of anti-apoptotic genes [56].

PI3K also regulates actin cytoskeleton rearrangement. Studies have shown that constitutively active PI3K constructs can induce substantial remodeling of the actin cytoskeleton. This actin reorganization resulted in increased cell migration, which was also shown to occur with increased levels of myristoylated AKT [57]. PI3K mediated regulation of actin cytoskeletal and cell motility was further detailed in studies linking PI3K dependent actin reorganization to ARAP3, a protein containing multiple PH domains. The GAP domains of the ARAP3 protein function as an intermediary mediating the actin remodeling function