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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.

Keywords

Phosphatidylinositol-4,5-bisphosphate 3-kinase; PI3K; protein kinase B; AKT; endocytosis; exocytosis; membrane trafficking; caveolae; clathrin

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-bisphosphate (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.

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

Structure of PI3-kinase family members

Since its original discovery in immunoprecipitates of pp60^{v-src} and T/pp60^{c-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 membrane⁸⁻¹⁰. 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, p85 α and p85 β ; and a 110 kDa catalytic subunit known as p110 with p110 α , p110 β and p110 γ 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 stretches of amino acid residues^{5,15-17}.

Cloning of the PI3K catalytic subunit by protein microsequencing established that p110 is the epicenter of the PI3K activity, functionally organized in a heterodimeric p85-p110 complex^{18,19}. Mammalian p110 α 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.

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 gets activated in a multistep manner involving ligand-receptor mediated activation of PI3K which triggers the conversion of membrane bound PtdIns(3,4)P₂ (PIP₂) to PtdIns(3,4,5)P₃ (PIP₃), 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, Ca²⁺-calmodulin-dependent kinases or with members of the I κ B 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 survival⁴¹⁻⁴³. 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 the 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 kinases⁵⁰. 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 p110 subunit is activated by interaction with the 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 pathway that mediates its anti-apoptotic function at the mitochondria level via AKT mediated phosphorylation and activation of BAD proteins^{41,55}. Additionally, I κ B kinase phosphorylation via the AKT-dependent pathway leads to subsequent degradation of I κ B and subsequent NF- κ B nuclear translocation leading to activation of anti-apoptotic genes⁵⁶.

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 AKT⁵⁷. 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 of the PI3K/AKT pathway^{58,59}. Integrins act to regulate cell motility during cell migration and invasion by dynamically regulating actin rearrangement via signaling through integrin receptors and downstream effectors like integrin-linked kinases (ILK). Studies have shown that this actin reorganization function of integrin signaling is mitigated in a PI3K and AKT-dependent manner since abolishing AKT activity disrupts cell migration and invasion triggered by integrins⁶⁰. PI3K signaling has also been shown to participate in PDGF-mediated actin rearrangement and cell motility⁶¹. Furthermore, the interaction of PI3K with the Rho family of small GTPases provides another mechanism for PI3K signaling regulation in Rac and Cdc42 mediated remodeling of the actin cytoskeleton, which can in turn, regulate vesicular endocytosis of adenoviral particles⁶². These studies reinforce the concept that PI3K signaling and its downstream targets, particularly AKT, can regulate actin cytoskeletal remodeling and change intracellular vesicle trafficking.

Endocytosis and its regulation by PI3K signaling

Endocytosis can be defined as the regulated formation of membrane bound vesicles at the plasma membrane that can then traverse through the cytosol to fuse with a target organelle. Every eukaryotic cell displays endocytic mechanisms that are essential for maintaining cellular homeostasis which then contribute to cellular functions including the transmission of neuronal and proliferative signals, internalization of nutritive materials and communication with the external cell milieu defense mechanism against invading pathogens⁶³. PI3K has been shown in many studies to regulate multiple endocytotic processes (**Figure 1**). The essential role of PI3-kinase signaling in intracellular vesicular trafficking was established in studies related to VPS34P, a yeast homolog of PI3K and its importance in protein sorting in the yeast vacuole^{5,64}. The most common endocytic pathways through which macromolecular complexes gain entry into eukaryotic cells include

actin-dependent phagocytosis, clathrin-dependent coated vesicle formation and internalization, and clathrin-independent or caveolae-dependent internalization.

Recent studies have shown that PI3K plays an important role in regulating both early and late stages of glucose induced endocytosis upon insulin release. Activation of peripheral PI3K via glucose stimulation generates PIP3 that helps to recruit an Arf6 guanine-nucleotide-exchange factor (ARNO). Peripherally accumulated ARNO acts to convert the GDP form of Arf6 to its active GTP bound form that contributes to the formation of clathrin coated pits in the presence of AP-2 adaptor proteins. The final step of this early excision process occurs in the presence of dynamin-2. Additionally, local accumulation of ARNO recruits EP164 that arrests Rab27a in its GDP bound form to promote the concluding step of endocytosis after scission. Given the importance of endocytosis in glucose induced insulin release, PI3K is a critical modulator of some key aspects of diabetes disease progression⁶⁵.

This role for PI3K signaling is supported by a recent study that shows a direct effect on the metabolic state in relation to insulin sensitivity. Liver hepatocytes demonstrate regulation of class III PI3K via insulin stimulation that functions to modulate insulin receptor (IR) trafficking and its lysosomal degradation. Downregulation of Vsp15, a regulatory subunit of class III PI3K, leads to compromised internalization and degradation of IR and affects overall trafficking⁶⁶. This study emphasizes that PI3K is a critical player in regulation of vesicle trafficking and a potential target for the treatment of metabolic conditions involving aberrant insulin signaling. Additional studies in hepatocytes expand on the role of specific PI3K isoforms in vesicle trafficking during glucose uptake. The PI3K-C2 γ isoform from the class II family exerts control over insulin metabolism by association with Rab5-GTP. Integration of PI3K-C2 γ into Rab5 loaded endosomal vesicles leads to the activation of the Akt2 isoform to trigger the synthesis of glycogen synthase (GS) that then contributes to downstream phosphorylation of GSK3⁶⁷. Parallel studies on other class II PI3K isoforms, such as PI3K-C2 β , further strengthen the link between this pathway and insulin signaling. Surprisingly, inactivation of the kinase property of this particular PI3K isoform led to enhanced insulin sensitivity that provided protective effects against high fat diet induced liver steatosis⁶⁸. These studies all point to an integral role of various PI3K isoforms in control of vesicle trafficking and related metabolic activities through regulation of the insulin-mediated signaling axis.

Phagocytosis allows a cell to engulf and internalize large particles, a process that is common in phagocytic protozoa. Phagocytic macrophages are an integral component of the mammalian immune system^{69,70}. Various phagocytic signals induce actin reorganization at the site of particle-receptor interaction to facilitate extension of pseudopod-like processes that can engulf bound particles^{71,72}. Macrophage induced phagocytosis of apoptotic cells constitutes an important mechanism to alleviate inflammatory responses and initiate tissue remodeling. The involvement of PI3K in the process of phagocytosis was deduced from studies in murine models of bone marrow-derived macrophages demonstrating that in the presence of PI3K inhibitors wortmannin and LY2944002 there was significant inhibition of apoptotic cell phagocytosis⁷³. In addition to phagocytosis, PI3K has been linked to tyrosine kinase mediated macrophage chemotaxis and also directly involved in regulating Fc γ R-mediated phagocytosis. Fc γ R-mediated phagocytosis was shown to be upregulated with

higher accumulation of PIP3 that correlated with the enhanced activity of multiple isoforms of PI3K demonstrating the importance of PI3K signaling in Fc γ R receptor phagocytosis⁷⁴⁻⁷⁶. Additionally, a recent study supports the involvement of the PI3K cascade in the engulfment of large particles via inhibition of Rac and Cdc42 through the action of RhoGAPs in comparison to smaller particles that occurs independent of the pathway. PI3K and its second messenger products promote translocation of RhoGAPs to the site of larger phagocytic curvatures that lead to localized actin destabilization and consequent internalization⁷⁷. These studies have helped to define that role of PI3K signaling in the process of phagocytosis.

Clathrin-dependent endocytosis is another endocytotic mechanism that principally functions in the internalization of ligand-receptor complexes and extracellular macromolecules in many eukaryotic cell types. The process is mediated through the invagination of clathrin lattices at the surface to form the adaptor coated vesicles known as clathrin-coated vesicles (CCVs)⁷⁸. PI3K has been shown to play a regulatory role in mediating selective clathrin-dependent endocytic processes. The PI3K C2 α isoform has been shown to be enriched in CCVs with clathrin acting to facilitate the catalytic activity of class II PI3K C2 α . PI3K C2 α directly affecting clathrin-mediated endocytosis and protein trafficking in trans-Golgi compartments⁷⁹. The influence of PI3K signaling in clathrin-dependent endocytosis is bolstered by studies demonstrating that internalization of β_2 -adrenergic receptor (β_2 -AR) endocytosis following agonist stimulation is dependent on interaction with PI3K machinery. A direct interaction between β_2 -AR and PI3K components was observed using immunoprecipitation techniques. Disrupting the interaction between the β -adrenergic receptor kinase1 (β ARK1) and PI3K by overexpression of the PIK accessory domain of PI3K resulted in a retarded interaction between PI3K and β_2 -AR with a marked decrease in receptor endocytosis. The specificity of the interaction was further documented by studies that showed no effect of transferrin internalization with overexpression of the accessory PIK domain⁸⁰. PI3K regulation of clathrin-mediated endocytosis was also supported by studies showing that hypo-phosphorylation of PI3K/p85 by EGF resulted in a marked defect in EGF-receptor endocytosis and trafficking in HeLa cells. The p85 subunit that can be tyrosine phosphorylated downstream of EGF stimulation was shown to be hypo-phosphorylated in HeLa cells that displayed defective endocytotic activity. The delayed activation of p85-PI3K may strengthen the fact that the regulatory role of PI3K in receptor trafficking occurs at the late stage of endocytosis rather than during early receptor internalization events⁸¹.

In contrast to clathrin-dependent mechanisms, clathrin-independent modes of endocytosis are relatively a new concept and are thought to be a major alternative route for viruses and extracellular materials to gain entry into the cells^{82,83}. The clathrin-independent endocytotic process can be categorized via their different routes; small GTP Rho-A/cdc42 dependent, Arf6-dependent and caveolae-mediated pathways. RhoA regulates lipid raft mediated endocytosis in a dynamin-dependent process, while Cdc42 does so in a dynamin-independent manner⁸⁴. Recent studies indicate that PI3K signaling is important in mediating some of these clathrin-independent endocytic processes. The importance of the role of PI3K in mediating clathrin-independent endocytosis was established in studies using mouse embryonic fibroblasts (MEFs) showing that induced PI3K signaling inhibition significantly attenuated dextran uptake whereas transferrin uptake was unaltered. Ras-mediated activation

of PI3K is important for the entry of influenza viruses that occurs in a clathrin-independent fashion and in the subsequent inhibition of the interaction between PI3K signaling and Ras. This results in decreased viral entry as confirmed with the suppression of the PI3K pathway by pharmacological inhibition. Not only viral entry but intracellular viral transport from early to late endosomes was severely affected by compromised PI3K signaling⁸⁵. Recent studies have shown that clathrin-independent endocytosis of interleukin 2 (IL-2) receptors occurs in a PI3K dependent manner since mutation of the Rac1 binding site in PI3-kinase compromises IL-2 receptor endocytosis. Association of IL-2 receptor with p85 α subunit leads to the activation of PI3K that induces activation of downstream partners such Vav2 and Rac1. This creates a feedback loop that further activates the Rac1/Pak1/N-WASP cascade that collectively functions to induce actin assembly to initiate receptor endocytosis⁸⁶. Internalization of major histocompatibility complex (MHC) class I proteins and integrins involves an arf6-dependent endocytic pathway mostly via a dynamin-independent fashion^{87,88}. Regulation of the Arf6-dependent endocytic process by PI3K signaling was demonstrated in studies showing that the early multifunctional gene product of HIV-1 virus, Nef, downregulates MHC class I molecule endocytosis from the plasma membrane to the trans-Golgi network in a PI3K dependent manner⁸⁹.

PI3K also regulates endocytosis through membrane invaginations known as caveolae. These 50-100 nm membrane invaginations appear in the plasma membrane of many cell types and contribute to regulation of many signaling functions including receptor internalization, as well as entry of viruses and other macromolecular structures^{90,91}. These structures are enriched for caveolin proteins and act as an organization center for signaling molecules which regulate their function in a cell specific and stimulus dependent manner^{92,93}. Caveolae harbor the molecular machinery associated with the PI3K signaling cascade⁹⁴. Compartmentalization of PI3K in caveolae has functional consequences since overexpression of caveolin-1 significantly upregulates activation of PI3K and its downstream effector AKT. Upregulation of AKT activation and its further downstream target GSK-3 α/β by caveolae resident proteins provide protective effects against arsenite treatment and increased cell survival^{95,96}. Additionally, caveolin-1 can regulate embryonic stem cell growth in response to EGF in a PI3K/AKT dependent manner. Major enrichment of EGFR was observed in caveolae in response to EGF stimulation that allows caveolin-1 to exert its proliferative effect through the activation of PI3K/AKT and ERK1/2 pathways. The proliferative role of caveolin-1 was confirmed with targeted knockdown of caveolin-1 that significantly attenuated EGF mediated embryonic stem cell proliferation⁹⁷.

Although a considerable amount of literature defines direct interaction of PI3K with caveolae/caveolins, exactly how this interaction facilitates the caveolae-mediated endocytic processes is still considered to be an active area of investigation. Interestingly, PI3K mediated regulation of the endocytic process has been implicated in neuronal cell lines. These studies demonstrate that the neurotrophin, nerve growth factor (NGF), mediates neuronal survival and differentiation via TrkA receptors in a PI3K dependent manner. PI3K induces TrkA receptor internalization which enables it to bind NGF and MAP kinase signaling proteins to mediate cell survival functions. In that study, PI3K endocytosis was also speculated to regulate extracellular signal-regulated kinase (ERK) and members of the MAP kinase cascade⁹⁸.

Exocytosis and its regulation by PI3K signaling

All eukaryotic cells possess inherent molecular mechanisms for exporting intracellular material or membrane bound vesicles into the extracellular space by a process aptly termed as exocytosis. The efflux of intracellular vesicles into the extracellular space depends on the transport of vesicles to the plasma membrane by molecular motors in which these vesicles fuse with the plasma membrane. This allows the vesicle contents to release into the extracellular space in an energy dependent manner. Previous studies categorized the exocytic process into two main branches; one that principally occurs constitutively as a vesicle approaches the plasma membrane and another more regulated form that requires specific stimuli, such as a rise in intracellular Ca^{2+} concentration, to trigger vesicular fusion and induce the process of exocytosis⁹⁹⁻¹⁰². A critical event in the exocytic process is membrane fusion, which requires involvement of protein machinery to mediate this fusion process. Membrane fusion in eukaryotic cells is mediated by several families of protein including SNAREs, Rab proteins, and Sec-1/Munc-18 proteins collectively known as the SM-proteins¹⁰³⁻¹⁰⁶. While PI3K can regulate endocytosis in multiple ways (**Figure 1**), the role of PI3K signaling exocytosis is best exemplified through its regulatory role in trafficking vesicles containing GLUT4 receptors to the plasma membrane following insulin stimulation¹⁰⁷⁻¹⁰⁹. Studies in 3T3-L1 cells indicate that the PI3K pathway acts through its downstream target serine/threonine kinase AKT to facilitate glucose transport in response to insulin stimulation. In 3T3 cells, the canonical glucose transport receptors, GLUT4 receptors, remained sequestered in the perinuclear region and also in smaller cytoplasmic vesicles localized near the plasma membrane. This creates a favorable environment by reducing the transport distance traversed by the receptors in order to exocytose from the membrane. The sequestered vesicles translocate and fuse with the plasma membrane to initiate exocytosis of the GLUT4 receptors. The early step that triggers this process involves activation of PI3K and generation of a substantial pool of PIP3. PIP3 lipids provide the docking site for PH domain harboring proteins and activation of AKT by phosphorylation at residues Thr308 by PDK1 and Ser473 by mTORC2. The series of phosphorylation on AKT leads to its activation thus promoting GLUT4 vesicular transport towards the membrane. This role of PI3K/AKT in GLUT 4 receptor trafficking has been deduced from studies involving active AKT mutants and knockout mouse models¹⁰⁹⁻¹¹¹.

Insulin induced activation of PI3K also altered glucose transporter GLUT4 recycling. Treatment of cells with the PI3K inhibitor wortmannin, significantly attenuated GLUT4 exocytosis, thus establishing a positive regulatory role for PI3K pathway in the GLUT4 receptor recycling process¹¹². The role of AKT1 in stimulating GLUT4 translocation to the plasma membrane in response to insulin has been shown to occur in cells of skeletal muscle origin and adipocytes^{113,114}. Recent studies dissecting the precise step at which PI3K exerts its regulatory control over GLUT4 receptor exocytosis reveal that it regulates the fusion of the receptor loaded vesicles with the plasma membrane¹¹⁵. PI3K demonstrated both positive and negative regulation of insulin release. The p110 γ subunit of Type I PI3K is required to maintain a ready pool of insulin loaded vesicles for the recruitment of insulin granules in the islet β -cells for release upon exocytic stimulation¹¹⁶. Similarly, PI3K C2 α , a class II PI3K isoform, exerts signaling effects on insulin secretion from pancreatic β -cells by promoting

insulin loaded granules in rat insulinoma cells. This does not affect the population of insulin granules at the plasma membrane nor the fate of other signaling proteins involved in insulin secretion¹¹⁷. In addition to this role in membrane fusion, PI3K is also known to contribute to glucose homeostasis by regulating β -cell gene expression. This has been confirmed by studies using cell lines with complete knockout of insulin receptor and insulin receptor substrate. Cellular deficiency of insulin signaling machineries dampens the expression of the insulin signaling proteins and obliterates downstream signaling mediated through the PI3K pathway^{118,119}.

PI3K signaling regulates additional exocytic cellular events. The link between exocytosis and PI3K signaling is important to nitric oxide (NO) mediated resistance to hypoxia in isolated hepatocytes. The beneficial effect of nitric oxide to combat hypoxic conditions was abolished in the presence of the PI3K inhibitor, wortmannin. During exposure to a hypoxic environment, NO release triggers stimulation of two bifurcated signaling cascades, one involving Ras, a G-protein receptor mediated signaling and the other occurs through a cyclic GMP route. The activation of these dual pathways acts to converge on the stimulation of PI3K that can enhance lysosomal trafficking events to the plasma membrane. The result of emptying of lysosomal content on the plasma membrane leads to an increased population of vacuolar H⁺-ATPase that helps to stabilize the cellular alteration of sodium and pH and in the process contribute to combat hypoxic conditions¹²⁰.

Concluding Remarks

Years of intensive study of the PI3K signaling cascade have linked this pathway to regulation of myriad cellular functions and established this pathway as a central molecule in multiple cellular signaling transduction networks. This review focuses on the importance of PI3K mediated signaling in the regulation of specific endocytic and exocytic processes. The process of intracellular vesicular trafficking is a key cellular function that facilitates many of the important cell functions. Delineating the molecular basis of PI3K signaling mediated regulation of endocytic and exocytic processes is crucial to laying the foundation for development of therapeutic candidates to address various pathophysiological conditions resulting from aberrant intracellular vesicular trafficking. While targeting the PI3K pathway may be an attractive approach to modulate vesicular trafficking in various pathological states there are concerns with such efforts because of the multiple cellular functional regulated by this signaling cascade. Direct targeting of PI3K function could lead to detrimental effects ranging from cellular toxicity to oncogenesis. Thus, increasing our understanding of additional aspects of the PI3K signaling pathway that serve more specific roles in particular cells or during disease progression could provide new targets that could avoid concerns about modulating the activity of a central regulator protein such as PI3K. Future studies will continue to dissect how PI3K signaling controls vesicle trafficking by resolving additional factors that mediate this process both upstream and downstream of the PI3K protein.

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Figure 1. PI3-kinase signaling regulates various vesicular trafficking events during endocytosis and exocytosis

The regulated release of intracellular content into the external milieu can be regulated by activation of PI3K and its downstream effectors. Internalization of foreign materials through the process of endocytosis constitutes an important mechanism that allows the cell to maintain key functions. PI3K signaling has been heavily implicated in the regulation of both clathrin-dependent and -independent modes of endocytotic internalization. PI3K also participates in regulation of caveolae-mediated trafficking events and in spontaneous membrane ruffling to induce phagocytosis. The dependence of various endo- and exocytic processes on the PI3-kinase pathway demonstrates its importance as a critical regulator of key physiological processes.