REVIEW

Phosphoinositide 3-kinases as a common platform for multi-hormone signaling

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Abstract

In multicellular organisms, concerted actions of different tissues are regulated inside single cells by signal transduction mechanisms that, subsequently to hormones sensing, trigger intracellular responses. In recent years, increasing evidence indicates phosphoinositide 3-kinases (PI3K) as crucial signal transducing elements that regulate communication across the plasma membrane. PI3K generate lipid secondary messengers that trigger a plethora of intracellular responses ranging from metabolic regulation to cell proliferation, survival, and

migration. The growing number of hormones that relay signals by activating PI3K suggests not only multiple roles of these enzymes in the regulation of different physiological responses but also a way by which common reactions can be stimulated by different inputs. This review will thus focus on the different pathways that converge on PI3K activation, with particular attention to the paradigmatic PI3K involvement in insulin signaling.

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Signal transduction: a matter of amplifiers

Homeostasis of multicellular organisms requires precise communication between different cells. The paradigm of hormonal function defines a cellular source of signals and a definite target able to interpret and respond to the stimulation. Currently, multiple signaling mechanisms are known that, at the target site, detect extracellular cues, provide intracellular amplification, and trigger specific responses. Receptor stimulation triggers the production of second messenger molecules that intensify signal detection. Among these molecules, cyclic AMP, Ca^{2+} , and lipid derivatives are able to bind effector proteins and elicit cellular reactions. While cAMP and Ca^{2+} so far appear to bind a limited number of effector proteins (e.g. only protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac) are direct targets of cAMP), phosphorylated lipids of the phosphatidyl inositol (PtdIns) family are interacting with hundreds of different proteins through common specialized binding domains. This highlights the versatility of phosphoinositide phosphate signaling cascade and supports the view that PtdIns signaling is a crucial ancestral biological mechanism.

PtdInsPs and PI3K

Phosphoinositides

Whereas inositol and its phosphorylated derivatives are found in bacteria, it is only in eukaryotes that PtdIns and its phosphorylated products (phosphoinositides) appear as lipid components of cell membranes involved in signaling (Michell 2007). Phosphoinositides are present in yeast and they are likely evolved as a means to mark membranes and regulate organelle identity as well as vesicular trafficking (Di Paolo & De Camilli 2006). In contrast to other types of signaling molecules, PtdIns phosphates label the membrane–cytosol interface without diffusion from one isolated membrane to another, thus stably marking, for example, vesicles and organelles. Enzymes that produce or degrade phosphoinositides probably evolved as a spatially restricted machinery that specifically scores membranes, promoting segregation of different compartments and providing a code to identify the fate of vesicles in their intracellular traffic. Similarly, protein binding to phosphoinositides could have appeared to ensure detection of such code as well as achievement of the targeted membrane fusion necessary for vectorial vesicular traffic. A further wave of diversification, dated at least one billion years ago, probably led to the appearance of the signaling

It is now clear, for example, that class I PI3K phosphorylate *in vivo* PIP₂ to produce PIP₃ and that class III enzymes produce PtdIns3P from PtdIns (Engelman *et al.* 2006). Nonetheless, the activity of class II PI3K is still debated and probably they are involved in the production of both PIP₃ and PtdIns3P (Engelman *et al.* 2006). So far, though mounting evidences indicate that classes II and III PI3K might be involved in vesicular trafficking (Maffucci *et al.* 2003, Lindmo & Stenmark 2006), class I PI3K represent the best characterized type. Class I PI3K are usually heterodimers consisting of a catalytic subunit and an adaptor/regulator subunit. All class I catalytic subunits share substantial homology, show a molecular mass of ~110 kDa, and are collectively referred to as p110 subunits. Mammals possess four class I PI3K *p110* genes named *Pik3ca*, *Pik3cb*, *Pik3cg*, and *Pik3cd* that are, however, usually termed PI3K α , β , γ , and δ . While *Pik3ca* and *Pik3cb* are ubiquitously expressed, *Pik3cg* and *Pik3cd* are preferentially found in leukocytes, with the exception of *Pik3cg* being recently detected in the cardiovascular system (Hirsch *et al.* 2006). Proteins encoded by these genes share four regions of highest homology that comprises, from the N-terminal end, the domain for binding to the small GTPase Ras, the lipid-binding C2 domain, the PIK domain (a helical motif common to all PI3K), and the catalytic domain. Of these four enzymes, only the structure of PI3K γ has been resolved (Walker *et al.* 1999). The catalytic pocket of PI3K γ shows a two-lobe organization, where the first is involved in ATP binding (through a fundamental lysine residue found in all PI3K) and the second is more involved in substrate specificity (Walker *et al.* 1999). This catalytic cleft is able to specifically accommodate small molecule inhibitors that like wortmannin and LY294002 effectively block enzymatic activity of all PI3K (Walker *et al.* 2000). Nonetheless, the presence in the pocket of residues specific to the PI3K γ isoform allows the generation of selective inhibitors able to act more specifically on PI3K γ (Camps *et al.* 2005). While binding of PI3K γ to Ras has been described at atomic resolution (Pacold *et al.* 2000), an in-depth description of the structural details leading to the interaction of p110s with their adaptor/regulator subunit is still missing.

Two class I PI3K types

The presence of different families of adaptor/regulator subunits defines two subgroups in the family of class I PI3K. PI3K α , β , and δ enzymes bind to adaptor/regulators of the so-called p85 family and embody the IA subgroup (Fig. 2). Of these p85 adaptor/regulators, three genes named *Pik3r1*, *Pik3r2*, and *Pik3r3* are found in mammals. *Pik3r1* can be expressed in three splice variants that encode p85 α , p55 α , and p50 α respectively; on the other hand, *Pik3r2* and *Pik3r3* are known to present only one transcript and encode p85 β and p55 γ respectively. While p85 α and p85 β are ubiquitously expressed (Gout *et al.* 1992), p50 α and p55 α are present in fat, muscle, liver, and brain (Antonetti *et al.* 1996, Inukai *et al.* 1996), and p55 γ is mainly expressed in the brain (Pons *et al.*

1995). All members of the p85 family contain a p110-binding region tethering them to a specific domain located at the N-terminal end of class IA p110s. In p85 proteins, the p110-binding region is sandwiched between two SH2 domains and these two regions bind to phosphorylated tyrosine residues in the YXXM motif of tyrosine-phosphorylated receptors or receptor-associated adaptor signaling molecules. This property defines a way of activation peculiar to class IA PI3K that generally act downstream tyrosine kinase receptors. In the currently accepted model of class IA activation, p110 subunits are constitutively bound to p85 and kept inactive in the cytosol. Upon receptor activation, p85 is recruited to tyrosine-phosphorylated motifs and this event not only liberates the p85-mediated constraint on PI3K catalytic activity (Fu *et al.* 2003) but also localizes the enzyme next to the plasma membrane in the proximity of its lipid substrate (Yu *et al.* 1998). In addition to controlling activity and localization of PI3K, p85 family members possess specific activities: for example, p85 can control cell cycle in a p110-independent way (Garcia *et al.* 2006). In addition, p85 α and β contain an SH3 domain that ensures interaction with intracellular protooncogenes and oncogenes like Cbl or breakpoint cluster region (BCR)/Abl (Ren *et al.* 2005).

The single member of class IB PI3K, PI3K γ , on the other hand, does not show an N-terminal p85-binding motif and interacts with the p101 (Stephens *et al.* 1997) and p84/87 adaptor/regulators (Suire *et al.* 2005, Voigt *et al.* 2005). PI3K γ is uniquely activated by G protein-coupled receptors (GPCR) and regulated by free G $\beta\gamma$ subunits of heterotrimeric G proteins usually of the G_i subtype (Hirsch *et al.* 2006). Interaction with G $\beta\gamma$ can be direct and sufficient to trigger enzymatic activity, but the presence of the non-catalytic adaptor/regulators facilitates this event by tethering PI3K γ to the plasma membrane (Brock *et al.* 2003). Interestingly, while PI3K γ is uniquely coupled to GPCR, the feature of being activated by G $\beta\gamma$ is probably shared with the class IA PI3K β which can be promiscuously activated by both tyrosine kinase receptors and GPCR (Murga *et al.* 2000). On the other hand, like class IA PI3K, PI3K γ binds GTP-loaded Ras and this interaction can contribute to its activation, though to a limited extent (Suire *et al.* 2002). The binding of PI3K γ to Ras has, however, a crucial role *in vivo* in the triggering of reactive oxygen species (ROS) production (Suire *et al.* 2006). Interestingly, PI3K γ is not only acting like an enzyme but also possesses a scaffolding activity that controls the activation of the phosphodiesterase 3B, thus linking PI3K γ to the modulation of cAMP destruction and compartmentalization (Patrucco *et al.* 2004, Hirsch *et al.* 2006).

PIP₃ phosphatases

The product of class I PI3K activity is the substrate for a series of phosphatases that specifically remove phosphate groups from the inositol ring of PIP₃. For example, hydrolysis of the phosphate in the D3 position is achieved by phosphatase and tensin homolog deleted on chromosome ten (PTEN) and is

Figure 2 Class I PI3K family. Four different class I PI3K are expressed in mammalian cells; depending on their mechanisms of activation, they can be divided into two subtypes: class IA PI3K (PI3K α , β , δ) are activated mainly by membrane-bound tyrosine kinase receptor and class IB PI3K γ is exclusively activated by GPCR generally of the G_i subtype. Class IA PI3K bind to the adaptor subunit p85, which, in turn, is recruited by its phosphotyrosine binding domain to phosphorylated receptors or adaptor proteins like IRS (insulin receptor substrate). Class IB PI3K γ binds to adaptor/regulators like p101 or p84/87 and to free G $\beta\gamma$ liberated by active GPCRs. All class I PI3K phosphorylate PIP₂ to produce PIP₃, a secondary messenger membrane lipid that functions as a docking site to a large number of proteins containing the pleckstrin homology (PH) domain. These effectors in turn control the different cellular responses summarized in Fig. 3.

thought to terminate PI3K-mediated signaling (Cully *et al.* 2006). On the other hand, SH2-containing inositol 5'-phosphatase (SHIP) proteins are able to remove the phosphate in the D5 position, thus indicating that one mechanism of PtdIns(3,4)P₂ appearance is due to PIP₃ degradation (Kalesnikoff *et al.* 2003).

PI3K downstream effectors

Class I PI3K products exert their large number of biological functions because of their ability to function as docking sites for proteins that contain specific lipid-binding domains such as the pleckstrin homology (PH) domain. Interestingly, PH domain is more abundant in the human proteome, reaching the 11th position of the most common sequences (Lemmon 2007). PH domains are found with different sequence organization and specificities: some, like, for example, that found in Akt, specifically bind PIP₃ but others, like the motif found in phospholipase C (PLC) δ 1 (Lemmon *et al.* 1995), selectively interact with PIP₂. Although not all PH domains containing proteins bind to phosphoinositides with sufficient strength for this to be of functional importance, the high number of proteins with such a domain highlights the complexity of signaling events downstream PI3K. A number

of other protein domains are also able to bind to phosphoinositides 3-phosphate. For example, of note is the binding of the phox homology domain (PX; Xu *et al.* 2001) and domain present in Fab1, YOTB, Vac1 and EEA1 (FYVE) domains to PtdIns3P (Kutateladze 2006). For the sake of clarity, only the best characterized elements binding PIP₃ will be further considered (Fig. 3).

One of the most studied signaling events triggered by PI3K is the activation of a number of serine/threonine kinases of the protein kinases A, G and C (AGC) family such as PDK1 and the three isoforms of PKB/Akt. Binding to PIP₃ triggers the activation of PDK1 that is then able to phosphorylate the threonine 308 of a PIP₃-bound PKB/Akt. For the full activation of PKB/Akt kinase, a second phosphorylation event in a hydrophobic loop containing serine 473 is necessary. Though phosphorylation of this latter residue depends on PIP₃ production, the exact mechanism involved is still elusive. Recent reports, however, indicate that the rictor-mTOR (TORC2) protein complex is the long-sought key regulator of this process (Sabatini 2006). Active PKB/Akt is able to phosphorylate a vast number of proteins (at least 75 substrates have so far been described), thus representing a key effector of PI3K signaling. Among the best characterized substrates is glycogen synthase kinase 3 (GSK3), an enzyme

Figure 3 Signal transduction pathways downstream PI3K. The product of PI3K activity, PIP₃, serves as a docking site for a large number of PH domain (in brown) containing proteins (listed in circle around PIP₃). These effector proteins represent the front line of PIP₃-dependent signaling. Further signal amplification is achieved by their activation and inhibition of multiple more specialized downstream targets, eventually producing specific biological responses. See text for explanation of the acronyms.

that is inhibited by PKB/Akt phosphorylation, thus allowing glycogen synthesis (Cross *et al.* 1995). In addition, PKB/Akt-mediated inhibition of GSK3 promotes cell proliferation by blocking phosphorylation-dependent degradation of cyclin D1 and Myc, two proteins required for cell cycle progression through the G₁ phase (Liang & Slingerland 2003). The second way of controlling cell proliferation is achieved by phosphorylating FoxO transcription factors (forkhead box O). Upon phosphorylation, these transcription factors are bound by 14-3-3 proteins and relocated to the cytoplasm where they can be degraded by the proteasome (Greer & Brunet 2005). This blocks p27^{kip} expression but supports transcription of cyclin D, thus facilitating cell cycle entrance (Schmidt *et al.* 2002). In the nucleus, signal termination of active PKB/Akt is achieved by the formation of a complex with the tumor suppressor promyelocytic leukemia (PML) that, by recruiting the phosphatases PP2A, causes PKB/Akt dephosphorylation and inactivation (Trotman *et al.* 2006). Signaling mediated by active PKB/Akt is crucial for promoting cell survival: PKB/Akt not only phosphorylates and inhibits the pro-apoptotic factor BCL2-antagonist of cell death (BAD) but also phosphorylates murine double minute2 (Mdm2) leading to p53 degradation (Engelman *et al.* 2006). On the other hand, PKB/Akt controls metabolism by promoting glucose

transporter 4 (Glut4) membrane targeting (Thong *et al.* 2005). Similarly, metabolic control is achieved by the FoxO-mediated inhibition of genes involved in liver gluconeogenesis (Barthel *et al.* 2005). Furthermore, PKB/Akt plays a crucial role in the control of protein synthesis by activating the raptor-mTOR (TORC1) complex (Wullschlegel *et al.* 2006). PKB/Akt phosphorylates and inactivates Tsc1 and 2 (tuberous sclerosis complex 1 and 2), two GTPase-activating proteins (GAP) that are able to inhibit the Rheb small GTPase. Upon release of the inhibitory action of Tsc1 and 2, active Rheb in turn binds and stimulates protein kinase activity of the TORC1 complex by still unclear mechanisms (Wullschlegel *et al.* 2006). TORC1 in turn phosphorylates and triggers S6K, a key regulator of S6 ribosomal protein, as well as other elements necessary for the initiation of mRNA translation such as eukaryotic initiation factor 4B (eIF4B), programmed cell death protein 4 (PDCD4), and eEF2K. TORC1 also directly phosphorylates 4E-binding protein (4E-BP) which releases its repressor activity on the mRNA 5' cap structure-binding factor eIF4E, thus triggering protein synthesis (Sonenberg & Pause 2006). More subtle roles of PKB/Akt have been reported in the control of cell contractility, due to an involvement in NO release through the phosphorylation/activation of NO synthase 3 (NOS3;

Dimmeler *et al.* 1999) or of extracellular Ca^{2+} entry because of a direct effect on L-type Ca^{2+} channel that leads to its plasma membrane exposure (Viard *et al.* 2004).

Although PKB/Akt is at the center stage of PI3K signaling, other well-characterized proteins can function as downstream targets of PIP₃ production. It is worth mentioning, for example, that PI3K can trigger the activity of PH domain containing tyrosine kinases Tec and Btk (Lindvall & Islam 2002) or the phospholipase PLC γ (Maffucci & Falasca 2007). In addition, a PH domain is found in most activators of small GTPases of the Rho and Arf families (Hawkins *et al.* 2006), thus explaining the role of PI3K in cytoskeletal remodeling, and membrane trafficking respectively. On the other hand, emerging evidence indicates that GAP inhibitors of Rho GTPases can also be regulated by PIP₃ binding and PI3K-mediated signaling (Di Paolo & De Camilli 2006). PI3K activity thus crucially controls the delicate balance between activating and inhibitory stimuli through, for example, the establishment of positive and negative feedback loops that help to transform shallow gradients of extracellular stimuli into robust cellular responses.

PI3K as a common platform for hormonal signal transduction

PI3K influence a variety of cellular functions, depending on the nature of the target cell and on the multiple signals impinging on a cell at a given time. It has recently become clear that PI3K are implicated in the signal transduction pathways of a wide variety of hormones. Because of the rapid enrichment of the field, this review shall focus on a few paradigmatic cases covering two distinct classes of hormones: peptides and steroids. Most peptide hormones have been reported to trigger PI3K activity, but only for a few like angiotensin II (Ang II), leptin, and insulin, specific knowledge of PI3K-dependent biochemical mechanisms, PI3K isoforms involved, or PI3K-mediated specific biological responses is sufficiently characterized. Similarly, amine and lipid hormones can trigger PI3K activity through their receptors but often with poorly characterized mechanisms. Unexpectedly, however, the PI3K-dependent signaling branch of steroid hormones like estrogen has been extensively analyzed and found to provide new concepts for their function.

PI3K and extranuclear estrogen receptor (ER) signaling

Estrogen actions are mediated by a mixture of both directly controlled gene expression (the so-called 'genomic or classical' action) and regulation of cell signaling/phosphorylation cascades, referred to as 'non-genomic or extranuclear' action. It is becoming increasingly evident that ER extranuclear signaling involves PI3K and might play central roles in controlling cell proliferation and survival (Fig. 4). Estrogen non-genomic effects on PI3K pathway might indeed be caused by direct interaction of the ER with PI3K (Migliaccio

et al. 1996, Castoria *et al.* 2001), in a process that involves the p85 regulatory subunit binding to ER and a subsequent ER-dependent PKB/Akt activation (Simoncini *et al.* 2000, Castoria *et al.* 2001, Sun *et al.* 2001). An alternative ER-mediated mechanism for PI3K triggering can be found in a restricted number of breast cancer lines and depends on ER-induced ErbB2 activation that subsequently triggers PI3K recruitment/activation (Stoica *et al.* 2003). Through PI3K, ER also exerts its anti-apoptotic effects: in MCF7 breast cancer cells treated with 17 β -estradiol (E2), modulator of non-genomic action of ER (MNAR) functions as a scaffold for ER α , cSrc, and p85 interactions, promoting PI3K activation and cell survival (Greger *et al.* 2007). Although ER controls PI3K activity, the opposite process has been described as well: in a number of epithelial cells, regulation of ER α -dependent transcriptional activity can be a target of PI3K signaling, even in the absence of estrogen, through a mechanism that involves PKB β /Akt2-mediated phosphorylation of ER (Sun *et al.* 2001).

The finding that ER can activate PI3K in normal and transformed cells suggests that this pathway might be involved in cancer growth. In agreement with this view, Sun *et al.* (2001) show that elevated levels of Akt2 and PI3K can be detected in 32 out of 80 primary breast carcinomas that are in majority ER α -positive. Consistently, a recent study on 342 human breast tumor samples and tumor cell lines, at about equal frequency in tumor stages I–IV, reports the presence of *Pik3ca* mutations that are significantly associated with the expression of E and progesterone receptors (ER/PR; Saal *et al.* 2005). Taken together, these findings thus indicate a strong link between ER and PI3K pathways in human breast cancer.

This same signaling cascade is also found to play roles in ER-mediated control of cardiovascular functions (Fig. 4). Estrogen, acting through ERs, plays a critical role in the protection of the cardiovascular system by pleiotropic effects, including vasodilatation, preservation of vascular integrity, and enhancement of cardiomyocyte survival by prevention of apoptosis. Indeed, estrogen-induced direct binding of ER α to p85 increases PI3K activity and results in the activation of PKB/Akt, which in turn triggers eNOS-mediated NO production (Simoncini *et al.* 2000). The activation of NOS indeed reduces inflammatory reactions and protects from the effects of ischemia/reperfusion (Simoncini *et al.* 2000). Similarly, in an animal model of myocardial infarction, involving permanent coronary occlusion, E2-mediated activation of PI3K and PKB/Akt protects the heart by reducing both infarct size and myocyte apoptosis (Patten *et al.* 2004). Overall, the activation of the ER/PI3K/Akt/eNOS pathway thus explains, in cultured human endothelial cells as well as *in vivo*, in intact elastic and muscular arteries, the molecular mechanism underlying the long-known protective effects of estrogen on female cardiovascular system (Haynes *et al.* 2000, Guo *et al.* 2005).

On the other hand, this signaling pathway might modulate cardiovascular responses to hormones, like Ang II, that might play a role in heart failure and stroke. ER efficiently reduces

Figure 4 Extranuclear signaling of the estrogen receptor. Upon binding to its ligands, like 17 β -estradiol (E2), the estrogen receptor (ER) not only activates the classic signaling pathway resulting in modulating gene transcription in the nucleus but also triggers PI3K activation in the cytoplasm, by directly binding to the p85 subunit. Listed are some of the biological effects of ER-dependent PI3K activation.

Ang II- or endothelin 1 (ET1)-induced hypertrophy of individual cardiomyocytes, resulting in a smaller cell surface area and decreased cytoskeletal protein expression. This effect is mediated by the PI3K-dependent activation of MCIP1, a negative regulator of the NFAT transcription factor involved in cardiac hypertrophy (Pedram *et al.* 2005).

PI3K in Ang II signaling

PI3K might also play a role in the signaling pathways triggered by Ang II itself, the peptide hormone backbone of the rennin-angiotensin system that controls blood pressure homeostasis as well as myocardial cell growth. Ang II and its GPCRs (AT receptors) play crucial roles by mediating smooth muscle growth, ROS production, and changes in vascular contractility. Indeed, the PI3K branch of Ang II-mediated signaling in vascular smooth muscle cells (VSMCs) is considered of increasing importance. For example, inhibition of PI3K activity with either LY294002 or wortmannin decreases AT1-receptor-stimulated activation of PKB/Akt (Takahashi *et al.* 1999), mTOR, and p70-S6 kinase as well as VSMC proliferation and gene expression (Saward & Zahradka 1997). Although Ang II regulates cell growth through the independent activation of PI3K and Erk1/2

pathways, it appears that both events are required for Ang II-induced proliferation of rat aortic smooth muscle cells (Dugourd *et al.* 2003).

In addition, Ang II controls vascular modulation of voltage-gated L-type Ca^{2+} channels by PI3K that thus appear as crucial regulators of Ca^{2+} entry and vascular excitation-contraction coupling. Ang II activates Ca^{2+} entry by stimulating L-type Ca^{2+} channels through $\text{G}_{\beta\gamma}$ -sensitive PI3K in portal vein myocytes (Le Blanc *et al.* 2004). Although both PI3K γ and α are expressed in vascular myocytes, Ang II-induced stimulation of vascular L-type Ca^{2+} channel and increase in Ca^{2+} depends only on the PI3K γ isoform (Macrez *et al.* 2001, Quignard *et al.* 2001). Similarly to VSMC, in cardiomyocytes, Ang II-evoked activation of PI3K γ , but not of PI3K α , causes pathological cell growth (Naga Prasad *et al.* 2000). More recently, Vecchione *et al.* have demonstrated that PI3K γ plays a role in Ang II signaling *in vivo* (Fig. 5). Indeed, mice lacking PI3K γ are protected from hypertension induced by administration of Ang II. PI3K γ controls smooth muscle contraction via two distinct pathways: on the one hand, it triggers PKB/Akt activation and the subsequent entry of extracellular Ca^{2+} through L-type Ca^{2+} channels and, on the other hand, PIP_3 produced by PI3K γ is necessary to trigger the small GTPase Rac

Figure 5 The role of PI3K γ in Ang II-dependent signaling. Binding of Ang II to its receptors (AT) activates a series of PI3K-dependent effects triggering vascular smooth muscle cell growth and proliferation through activation of PKB/Akt, mTOR, and S6K. At the same time, PKB/Akt phosphorylates the β -chain of L-type Ca^{2+} channels, enhancing their plasma membrane exposure and open probability. Furthermore, PI3K-mediated activation of the small GTPase Rac triggers reactive oxygen species (ROS) production, causing vascular contraction and remodeling.

possibly through the recruitment of PH domain containing GTP exchange factors (GEF). The activation of Rac, in turn, represents a critical step in the assembly at the plasma membrane and the subsequent activation of the NADPH oxidase complex triggering ROS production. These data indicate that PI3K γ is a key signaling molecule in the Ang II pathway and suggest that blocking PI3K γ function might be exploited to improve therapeutic intervention of hypertension (Vecchione *et al.* 2005).

PI3K-mediated effects of leptin

Leptin is another peptide hormone that modulates vascular tone as well as cell metabolism by stimulating PI3K. It binds the long form of leptin receptor (LRb) and triggers the tyrosine kinase JAK, eventually controlling STAT transcription factors and gene expression (Munzberg & Myers 2005). The way by which leptin also stimulates PI3K is not yet precisely defined, although different studies suggest JAK2-dependent and -independent mechanisms that trigger p85 binding to tyrosine-phosphorylated insulin receptor substrate (IRS, see below for description) adaptor proteins (Kellerer *et al.* 1997, Munzberg & Myers 2005, Mansour *et al.* 2006; Fig. 6). Although many effects of leptin are mediated through the central nervous system (for PI3K-mediated effects of leptin on the brain refer to review; Plum *et al.* 2005), this peptide can regulate metabolism through a direct action on tissues, such as pancreas and liver. In agreement with this finding, previous studies have shown that leptin activates PI3K in β -cells and hepatocytes (Wang *et al.* 1997, Harvey *et al.* 2000, Szanto & Kahn 2000). Moreover, in primary rat

hepatocytes, leptin induces PI3K-dependent activation of phosphodiesterase 3B, a cAMP-degrading enzyme, to suppress glucagon-induced cAMP elevation (Zhao *et al.* 2000). Leptin function in other tissues appears to similarly rely on PI3K signaling: for example, in VSMCs, leptin stimulates NOS activity and NO release, inhibiting the contractile response induced by Ang II, through mechanisms involving PKB/Akt and JAK2/STAT3 pathways (Rodriguez *et al.* 2007). On the other hand, it has recently become clear that leptin causes systemic effects on the regulation of immune function and some of these effects are PI3K mediated. Indeed, leptin causes a delayed apoptosis of mature neutrophils *in vitro*, through a signaling cascade involving PI3K (Bruno *et al.* 2005). Altogether, these indications suggest that leptin can induce PI3K signaling to influence different responses in distinct cell types.

PI3K signaling in insulin-mediated metabolic control

A growing body of evidence indicates that not only is PI3K activation a key step in insulin-mediated control of metabolism but also that PIP₃ production is fundamental for most biological responses exerted by insulin (Fig. 6). Activated insulin receptor (IR) triggers PI3K activity mainly by binding and phosphorylating adaptor proteins of the IRS family. IRS proteins are characterized by similar domains including a PH domain and a phosphotyrosine-binding domain, as well as by numerous tyrosine residues that can be phosphorylated by IR. Four different IRS proteins are

Figure 6 Class IA PI3K function in leptin (right) and insulin (left) induced signaling. Refer to text for details. Binding of free p85 subunits to insulin receptors/IRS complexes might be the cause of the paradoxical effects detected in mice that lack p85 genes. Excess of p85 could thus negatively regulate p110 function, which in the case of p85 reduction can eventually increase. Though a wealth of evidence points to a major role of PI3K α in insulin signaling, it is still unclear whether PI3K β has indeed no importance in the process.

expressed in mammals, though there are two additional IRS proteins detected in humans (IRS5/DOK4 and IRS6/DOK5) that are involved in insulin signaling but are not involved in PI3K activation (Cai *et al.* 2003). With a molecular weight ranging between 60 and 180 kDa, IRS proteins show differential tissue distribution as well as subcellular localization (Sun *et al.* 1997). While IRS-1 and IRS-2 are widely distributed (Araki *et al.* 1993, Sun *et al.* 1997), IRS-3 is limited to the brain, liver, adipocytes, and fibroblasts (Sciaccitano & Taylor 1997), and IRS4 is mainly expressed in embryonic tissues and adult muscle (Schreyer *et al.* 2003). Knockout mouse models indicate that IRS isoforms might play distinct roles: IRS-1-, IRS-2-, or IRS-4-deficient mice show defects on glucose homeostasis, with impaired glucose tolerance, increased serum triglycerides, and hepatic insulin resistance, but, in contrast, IRS-3 deficiency does not produce relevant effects on metabolism (Araki *et al.* 1994, Abe *et al.* 1998, Withers *et al.* 1998, Kubota *et al.* 2000).

IRS activity downstream the IR is tightly regulated by positive and negative regulation. IRS is activated by tyrosine phosphorylation and negatively regulated by protein tyrosine phosphatases (Hayashi *et al.* 2004, Gonzalez-Rodriguez *et al.* 2007). Other inhibitory mechanisms include blockade of their binding to the IR (Ueki *et al.* 2002a), or serine phosphorylation mediated by a PI3K-mediated negative feedback that involves IRS phosphorylation by S6K (Um *et al.* 2004).

Upon phosphorylation by active IR, IRS serves as a docking site for SH2 domains of class IA p85 regulatory/adaptor subunits that consequently recruit p110 enzymes next to the activated receptor. PIP₃ production in turn activates downstream effectors that control various metabolic processes such as glucose uptake, lipolysis inhibition, triglyceride formation, and glycogen synthesis. In these processes, the initiating event is the activation of PDK1 that, after PIP₃ binding, activates atypical PKC (aPKC), PKB/Akt, and S6K (Vanhaesebroeck & Alessi 2000). Among these downstream effectors, PKB/Akt stimulates blood glucose disposal, promotes synthesis of glycogen, and inhibits gluconeogenesis. Three different isoforms of PKB/Akt, encoded by different genes, are found in mammals (PKB α - γ or, alternatively, Akt1-3). All isoforms share a common structure including an N-terminal PH domain and a C-terminal catalytic domain. Recent data, obtained using knockout model mice, associate each PKB/Akt isoform with a specific function and indicate that not all are involved in insulin-mediated metabolic control. While PKB γ /Akt3 appears to be principally implicated in neuronal development (Tschoopp *et al.* 2005), PKB α /Akt1 is involved in growth and longevity (Chen *et al.* 2001, Cho *et al.* 2001a), but only PKB β /Akt2 is the main regulator *in vivo* of glucose homeostasis. Indeed, the absence of PKB β /Akt2 causes type 2 diabetes in both mice (Cho *et al.* 2001b) and humans (George *et al.* 2004).

Insulin, PI3K, and glucose metabolism

In adipocytes and muscle cells, PI3K-dependent activation of PKB β /Akt2 (and to a lesser extent of PKB α /Akt1) controls insulin-mediated glucose uptake by stimulating GLUT4 translocation from an intracellular compartment to the plasma membrane (Katome *et al.* 2003). GLUT4 trafficking is controlled by PKB/Akt-mediated phosphorylation of the RabGAP (Rab GAP) AS160 (Akt substrate of 160 kDa) that when phosphorylated is inhibited by binding to 14-3-3 protein (Ramm *et al.* 2006). Other PI3K-dependent mediators of glucose uptake are atypical forms of PKC, including PKC λ and PKC ζ isoforms. Overexpression of PKC λ and PKC ζ produces an increase in GLUT4 translocation, which is, conversely, inhibited by a dominant-negative mutant of PKC ζ (Bandyopadhyay *et al.* 1997).

Together with enzymes of class IA, PI3K of other classes might be involved in insulin-mediated glucose disposal. Recent experimental evidences suggest that insulin stimulation triggers the production of PtdIns3P, thus possibly involving either class II or III PI3K. For example, overexpression of myotubularins, the PtdIns3P phosphatases, reduces glucose uptake (Chaussade *et al.* 2003). PtdIns3P binds the PX and FYVE domains of proteins often involved in the control of vesicular trafficking. It is thus possible that PI3K other than class IA are involved in processes needed for plasma membrane fusion of cytoplasmic vesicles containing GLUT4 (Shepherd 2005). Interestingly, insulin-mediated PtdIns3P production appears insensitive to the action of wortmannin, thus excluding a role for class III enzymes but indicating a function of the wortmannin-insensitive class II PI3K-C2 α isoenzymes (Maffucci *et al.* 2003). In line with this view, PI3K-C2 α is activated in response to insulin-dependent (but PI3K-independent) activation of the small GTPase TC10, thus triggering PtdIns3P elevation necessary for full-scale GLUT4 plasma membrane translocation (Maffucci *et al.* 2003).

Another target of the class IA PI3K pathway is insulin-mediated inhibition of hepatic gluconeogenesis (Agati *et al.* 1998, Kotani *et al.* 1999). *De novo* synthesis of glucose is indispensable in a starved condition and, on the other hand, is dispensable when external resources are available. In this latter condition, insulin negatively regulates gluconeogenesis by suppressing the expression of key gluconeogenic enzymes through the PKB/Akt-mediated phosphorylation of the transcription factor FoxO1. After phosphorylation, FoxO1 is not able to translocate into the nucleus and consequently fails to activate transcription of various genes necessary for gluconeogenesis, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (Nakae *et al.* 2001).

Role of the regulatory subunit of PI3K

The crucial role of PI3K in insulin signaling suggests that its dysfunction may have deleterious effects on glucose homeostasis. Established lines of evidence indicate that PI3K signaling could be compromised by reducing expression of class IA PI3K

enzymes and adaptors in type 2 diabetic *ob/ob* mice (Folli *et al.* 1993, Kerouz *et al.* 1997) as well as humans (Goodyear *et al.* 1995, Bjornholm *et al.* 1997, Rondinone *et al.* 1997).

Gene deletion studies of p85 regulatory subunits have challenged this view and reported unexpected paradoxical effects of loss of p85 proteins. Mice lacking p85 α , one of the three splicing variants encoded by the *Pik3r1* gene, are viable but are hypoglycemic and show increased insulin sensitivity. However, this probably correlates with the up-regulation of the other *Pik3r1* splicing variants, p50 α and p55 α , in fat and muscle. This overexpression produces an elevation of PIP₃ levels in adipocytes, which facilitates the GLUT4 translocation to the plasma membrane (Terauchi *et al.* 1999). On the contrary, deletion of the *Pik3r1* gene, determining the ablation of all its products (p85 α , p55 α , and p50 α), results in perinatal lethality, extensive hepatocyte necrosis, enlarged skeletal muscle fibers, brown fat necrosis, and calcification of cardiac tissue. Loss of *Pik3r1* causes a substantial decrease in expression and activity of class IA PI3K catalytic subunits. Nonetheless, *Pik3r*-deficient mice are hypoglycemic and more insulin sensitive than controls because of a more active glucose transport in insulin-responsive tissues like fat and muscle (Fruman *et al.* 2000). Furthermore, hepatic deletion of *Pik3r1* produces similar paradoxical effects on PI3K activation/function. If, on the one hand, hepatic loss of *Pik3r1* improves insulin sensitivity in liver, muscle, and fat, on the other hand, it leads to severe reduction in PI3K activation, with a 60% decrease in total hepatic PI3K activity (Taniguchi *et al.* 2006). Nonetheless, the activation of PKB/Akt downstream PI3K is enhanced as a consequence of reduced activity of the phosphatase PTEN, thus suggesting a role of p85 in PTEN regulation (Taniguchi *et al.* 2006). Indeed, PTEN is a potent negative regulator of the insulin signaling and loss of PTEN in adipose tissue results in an increased insulin sensitivity and GLUT4 recruitment (Kurlawalla-Martinez *et al.* 2005).

The paradoxical enhancement of insulin signaling is also detected in mice lacking either p50 α /p55 α or p85 β . Although p50 α and p55 α are not required for survival, their loss produces a reduction in the insulin levels in the fasting state and an increase in glucose transport in isolated extensor digitorum longus (EDL) muscle and adipocytes (Chen *et al.* 2004). Similarly, mice lacking p85 β are viable and show hypoinsulinemia, hypoglycemia, and improved insulin sensitivity (Ueki *et al.* 2002b, 2003).

Taken together, these findings assign a positive and negative role to p85 regulatory subunits in the insulin signaling. In fact, p85 proteins are required for p110 stabilization but conversely free p85s exert negative effects on PI3K signaling. Therefore, a critical molecular balance between regulatory and catalytic subunits determines the optimal response of the PI3K pathway to insulin signaling (Ueki *et al.* 2002a). In physiological conditions, p85 is more abundant than the catalytic p110 subunit, producing a competition between p85 monomers and p85–p110 dimers. Monomers of p85 can thus inhibit PIP₃ production either by binding to phosphorylated IRS proteins (Ueki *et al.* 2002a) or by altering subcellular localization of p110/p85 dimers (Inoue *et al.* 1998).

Unbalancing p85 levels might thus compromise this regulatory mechanisms and lead to a paradoxical increase in PI3K activity. Finding of increased p85/55/50 protein expression, in mouse models of obesity as well as in type 2 diabetic patients, further confirms this view (Bandyopadhyay *et al.* 2005). Altogether, these findings suggest that p85 family members have a complex role in regulating PI3K-mediated insulin signaling that probably involves p110-independent activities yet to be fully understood.

Role of the catalytic subunit of PI3K

The use of no isoform-selective PI3K inhibitors has for long hindered the definition of the nature of the p110 catalytic subunits involved in insulin signaling. Nonetheless, recent reports using mutant mice as well as novel selective inhibitors suggest that different p110 subunits might play distinct roles. Although the lethal phenotype of mice lacking either p110 α (p110 α ^{-/-}) or p110 β (p110 β ^{-/-}) hampered the study of their relative role in the insulin signaling, analysis of insulin-mediated responses in heterozygous animals suggests that both proteins might be involved. Heterozygous mice lacking either PI3K α or β show normal responses to insulin; however, p110 α ^{+/-}/p110 β ^{+/-} compound heterozygotes display decreased insulin sensitivity (Brachmann *et al.* 2005). Nonetheless, heterozygous mice expressing a catalytically inactive PI3K α become insulin resistant with age. While young animals are normal, mice older than 6 months display glucose intolerance, hyperlipidemia, adiposity, as well as hyperglycemia and deregulate hepatic gluconeogenesis, thus assigning a fundamental role of PI3K α in insulin-dependent signaling (Foukas *et al.* 2006). Biochemical analysis further shows that only PI3K α is selectively recruited and activated by IRS proteins (Foukas *et al.* 2006). In agreement with this finding, the use of PI3K inhibitors with significant isoform selectivity shows that PI3K α is the major PI3K effector downstream the IR and that, in contrast, PI3K β maintains a marginal role (Knight *et al.* 2006). Nonetheless, inhibitor studies indicate that PI3K β influences PI3K α function providing a basal threshold of PIP₃ production that potentiates PI3K α activity, thus suggesting that PI3K β function might be still necessary for achieving full scale signaling responses (Knight *et al.* 2006). Further studies with mice possibly expressing a catalytically inactive PI3K β might help to address this issue.

How can different hormones activate PI3K but exert different biological responses?

Exploration of signaling mechanisms of most hormonal receptor agonists has led to the identification of PI3K activation with such a high frequency that it becomes legitimate to ask oneself if, in higher eukaryotes, receptor agonist that works independently of this pathway indeed exist. Furthermore, the fact that a multitude of extracellular stimuli relay PI3K activation poses important interrogatives concerning the mechanisms by

which selective extracellular cues trigger specific responses using the same signaling components. For example, although most hormones signal through PI3K to generally promote cell survival and proliferation, only insulin requires PI3K for the induction of glucose uptake. How this specificity is achieved is not yet understood. Nonetheless, a few speculations can be made: for example, different PI3K isoforms can be involved in determining when and where PIP₃ is produced inside a cell. Despite the wealth of information about the activation mechanisms of distinct PI3K isoforms, little is known about where PIP₃ is indeed located. Indications from studies in leukocytes suggest that subcellular localization of PIP₃ accumulation is fundamental for processes like directional cell migration (Hirsch *et al.* 2006). Consistent with this view, it is becoming increasingly suspected that compartmentalized pools of PIP₃ exist and that spatially restricted accumulation of second messenger molecules represents the key for solving the paradox of a single pathway eliciting selective distinct cellular responses. The finding that PI3K possess scaffold activity (Patrucco *et al.* 2004) suggests that signaling complexes involving these enzymes might be more complicated than initially thought and that such multi-molecular interactions might provide the mechanistic basis for localized and selective signaling. The rising expectation that our knowledge on PI3K signal transduction will soon provide new therapeutic tool for a vast number of pathologies (Camps *et al.* 2005) requires that further studies help to tackle these issues before encountering drawbacks and unwanted side effects.

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References

- Abe H, Yamada N, Kamata K, Kuwaki T, Shimada M, Osuga J, Shionoiri F, Yahagi N, Kadowaki T, Tamemoto H *et al.* 1998 Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *Journal of Clinical Investigation* **101** 1784–1788.
- Agati JM, Yeagley D & Quinn PG 1998 Assessment of the roles of mitogen-activated protein kinase, phosphatidylinositol 3-kinase, protein kinase B, and protein kinase C in insulin inhibition of cAMP-induced phosphoenolpyruvate carboxykinase gene transcription. *Journal of Biological Chemistry* **273** 18751–18759.
- Antonetti DA, Algenstaedt P & Kahn CR 1996 Insulin receptor substrate 1 binds two novel splice variants of the regulatory subunit of phosphatidylinositol 3-kinase in muscle and brain. *Molecular and Cellular Biology* **16** 2195–2203.
- Araki E, Sun XJ, Haag BL III, Chuang LM, Zhang Y, Yang-Feng TL, White MF & Kahn CR 1993 Human skeletal muscle insulin receptor substrate-1. Characterization of the cDNA, gene, and chromosomal localization. *Diabetes* **42** 1041–1054.
- Araki E, Lipes MA, Patti ME, Bruning JC, Haag B III, Johnson RS & Kahn CR 1994 Alternative pathway of insulin signalling in mice with targeted disruption of the *IRS-1* gene. *Nature* **372** 186–190.

- Bandyopadhyay G, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J & Farese RV 1997 Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. *Journal of Biological Chemistry* **272** 2551–2558.
- Bandyopadhyay GK, Yu JG, Ofrecio J & Olefsky JM 2005 Increased p85/55/50 expression and decreased phosphatidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. *Diabetes* **54** 2351–2359.
- Barthel A, Schmoll D & Unterman TG 2005 FoxO proteins in insulin action and metabolism. *Trends in Endocrinology and Metabolism* **16** 183–189.
- Berridge MJ 2005 Unlocking the secrets of cell signaling. *Annual Review of Physiology* **67** 1–21.
- Bjornholm M, Kawano Y, Lehtihet M & Zierath JR 1997 Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after *in vivo* insulin stimulation. *Diabetes* **46** 524–527.
- Brachmann SM, Ueki K, Engelman JA, Kahn RC & Cantley LC 2005 Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Molecular and Cellular Biology* **25** 1596–1607.
- Brock C, Schaefer M, Reusch HP, Czupalla C, Michalke M, Spicher K, Schultz G & Nurnberg B 2003 Roles of G beta gamma in membrane recruitment and activation of p110 gamma/p101 phosphoinositide 3-kinase gamma. *Journal of Cell Biology* **160** 89–99.
- Bruno A, Conus S, Schmid I & Simon HU 2005 Apoptotic pathways are inhibited by leptin receptor activation in neutrophils. *Journal of Immunology* **174** 8090–8096.
- Cai D, Dhe-Paganon S, Melendez PA, Lee J & Shoelson SE 2003 Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. *Journal of Biological Chemistry* **278** 25323–25330.
- Camps M, Ruckle T, Ji H, Ardisson V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Francon B *et al.* 2005 Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nature Medicine* **11** 936–943.
- Castoria G, Migliaccio A, Bilancio A, Di Domenico M, de Falco A, Lombardi M, Fiorentino R, Varricchio L, Barone MV & Auricchio F 2001 PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. *EMBO Journal* **20** 6050–6059.
- Chaussade C, Pirola L, Bonnafous S, Blondeau F, Brenz-Verca S, Tronchere H, Portis F, Rusconi S, Payrastré B, Laporte J *et al.* 2003 Expression of myotubularin by an adenoviral vector demonstrates its function as a phosphatidylinositol 3-phosphate (PtdIns(3)P) phosphatase in muscle cell lines: involvement of PtdIns(3)P in insulin-stimulated glucose transport. *Molecular Endocrinology* **17** 2448–2460.
- Chen WS, Xu PZ, Gottlob K, Chen ML, Sokol K, Shyanova T, Roninson I, Weng W, Suzuki R, Tobe K *et al.* 2001 Growth retardation and increased apoptosis in mice with homozygous disruption of the *Akt1* gene. *Genes and Development* **15** 2203–2208.
- Chen D, Mauvais-Jarvis F, Blüher M, Fisher SJ, Jozsi A, Goodyear LJ, Ueki K & Kahn CR 2004 p50alpha/p55alpha phosphoinositide 3-kinase knockout mice exhibit enhanced insulin sensitivity. *Molecular and Cellular Biology* **24** 320–329.
- Cho H, Thorvaldsen JL, Chu Q, Feng F & Birnbaum MJ 2001a Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *Journal of Biological Chemistry* **276** 38349–38352.
- Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI & Birnbaum MJ 2001b Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* **292** 1728–1731.
- Cross DA, Alessi DR, Cohen P, Andjelkovich M & Hemmings BA 1995 Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378** 785–789.
- Cully M, You H, Levine AJ & Mak TW 2006 Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nature Reviews. Cancer* **6** 184–192.
- Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R & Zeiher AM 1999 Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* **399** 601–605.
- Di Paolo G & De Camilli P 2006 Phosphoinositides in cell regulation and membrane dynamics. *Nature* **443** 651–657.
- Dugourd C, Gervais M, Corvol P & Monnot C 2003 Akt is a major downstream target of PI3-kinase involved in angiotensin II-induced proliferation. *Hypertension* **41** 882–890.
- Engelman JA, Luo J & Cantley LC 2006 The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature Reviews. Genetics* **7** 606–619.
- Folli F, Saad MJ, Backer JM & Kahn CR 1993 Regulation of phosphatidylinositol 3-kinase activity in liver and muscle of animal models of insulin-resistant and insulin-deficient diabetes mellitus. *Journal of Clinical Investigation* **92** 1787–1794.
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ & Vanhaesebroeck B 2006 Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature* **441** 366–370.
- Fruman DA, Mauvais-Jarvis F, Pollard DA, Yballe CM, Brazil D, Bronson RT, Kahn CR & Cantley LC 2000 Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. *Nature Genetics* **26** 379–382.
- Fu X, Aronoff-Spencer E, Backer JM & Gerfen GJ 2003 The structure of the inter-SH2 domain of class IA phosphoinositide 3-kinase determined by site-directed spin labeling EPR and homology modeling. *PNAS* **100** 3275–3280.
- Garcia Z, Silio V, Marques M, Cortes I, Kumar A, Hernandez C, Checa AI, Serrano A & Carrera AC 2006 A PI3K activity-independent function of p85 regulatory subunit in control of mammalian cytokinesis. *EMBO Journal* **25** 4740–4751.
- George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL *et al.* 2004 A family with severe insulin resistance and diabetes due to a mutation in AKT2. *Science* **304** 1325–1328.
- Gonzalez-Rodriguez A, Clampitt JE, Escibano O, Benito M, Rondonne CM & Valverde AM 2007 Developmental switch from prolonged insulin action to increased insulin sensitivity in protein tyrosine phosphatase 1B-deficient hepatocytes. *Endocrinology* **148** 594–608.
- Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ & Dohm GL 1995 Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *Journal of Clinical Investigation* **95** 2195–2204.
- Gout I, Dhand R, Panayotou G, Fry MJ, Hiles I, Otsu M & Waterfield MD 1992 Expression and characterization of the p85 subunit of the phosphatidylinositol 3-kinase complex and a related p85 beta protein by using the baculovirus expression system. *Biochemical Journal* **288** 395–405.
- Greer EL & Brunet A 2005 FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* **24** 7410–7425.
- Greger JG, Fursov N, Cooch N, McLarney S, Freedman LP, Edwards DP & Cheskis BJ 2007 Phosphorylation of MNAR promotes estrogen activation of phosphatidylinositol 3-kinase. *Molecular and Cellular Biology* **27** 1904–1913.
- Guo X, Razandi M, Pedram A, Kassab G & Levin ER 2005 Estrogen induces vascular wall dilation: mediation through kinase signaling to nitric oxide and estrogen receptors alpha and beta. *Journal of Biological Chemistry* **280** 19704–19710.
- Harvey J, McKay NG, Walker KS, Van der Kaay J, Downes CP & Ashford ML 2000 Essential role of phosphoinositide 3-kinase in leptin-induced K(ATP) channel activation in the rat CRI-G1 insulinoma cell line. *Journal of Biological Chemistry* **275** 4660–4669.
- Hawkins PT, Anderson KE, Davidson K & Stephens LR 2006 Signalling through Class I PI3Ks in mammalian cells. *Biochemical Society Transactions* **34** 647–662.
- Hayashi K, Shibata K, Morita T, Iwasaki K, Watanabe M & Sobue K 2004 Insulin receptor substrate-1/SHP-2 interaction, a phenotype-dependent switching machinery of insulin-like growth factor-I signaling in vascular smooth muscle cells. *Journal of Biological Chemistry* **279** 40807–40818.

- Haynes MP, Sinha D, Russell KS, Collinge M, Fulton D, Morales-Ruiz M, Sessa WC & Bender JR 2000 Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. *Circulation Research* **87** 677–682.
- Hinchliffe K & Irvine R 1997 Inositol lipid pathways turn turtle. *Nature* **390** 123–124.
- Hirsch E, Lembo G, Montrucchio G, Rommel C, Costa C & Barberis L 2006 Signaling through PI3Kgamma: a common platform for leukocyte, platelet and cardiovascular stress sensing. *Thrombosis and Haemostasis* **95** 29–35.
- Inoue G, Cheatham B, Emkey R & Kahn CR 1998 Dynamics of insulin signaling in 3T3-L1 adipocytes. Differential compartmentalization and trafficking of insulin receptor substrate (IRS)-1 and IRS-2. *Journal of Biological Chemistry* **273** 11548–11555.
- Inukai K, Anai M, Van Breda E, Hosaka T, Katagiri H, Funaki M, Fukushima Y, Ogiwara T, Yazaki Y, Kikuchi M *et al.* 1996 A novel 55-kDa regulatory subunit for phosphatidylinositol 3-kinase structurally similar to p55PIK is generated by alternative splicing of the p85alpha gene. *Journal of Biological Chemistry* **271** 5317–5320.
- Kalesnikoff J, Sly LM, Hughes MR, Buchse T, Rauh MJ, Cao LP, Lam V, Mui A, Huber M & Krystal G 2003 The role of SHIP in cytokine-induced signaling. *Reviews of Physiology, Biochemistry and Pharmacology* **149** 87–103.
- Katome T, Obata T, Matsushima R, Masuyama N, Cantley LC, Gotoh Y, Kishi K, Shiota H & Ebina Y 2003 Use of RNA interference-mediated gene silencing and adenoviral overexpression to elucidate the roles of AKT/protein kinase B isoforms in insulin actions. *Journal of Biological Chemistry* **278** 28312–28323.
- Kellerer M, Koch M, Metzinger E, Mushack J, Capp E & Haring HU 1997 Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* **40** 1358–1362.
- Kerouz NJ, Horsch D, Pons S & Kahn CR 1997 Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. *Journal of Clinical Investigation* **100** 3164–3172.
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B *et al.* 2006 A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell* **125** 733–747.
- Kotani K, Ogawa W, Hino Y, Kitamura T, Ueno H, Sano W, Sutherland C, Granner DK & Kasuga M 1999 Dominant negative forms of Akt (protein kinase B) and atypical protein kinase C lambda do not prevent insulin inhibition of phosphoenolpyruvate carboxykinase gene transcription. *Journal of Biological Chemistry* **274** 21305–21312.
- Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H *et al.* 2000 Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* **49** 1880–1889.
- Kurlavalla-Martinez C, Stiles B, Wang Y, Devaskar SU, Kahn BB & Wu H 2005 Insulin hypersensitivity and resistance to streptozotocin-induced diabetes in mice lacking PTEN in adipose tissue. *Molecular and Cellular Biology* **25** 2498–2510.
- Kutateladze TG 2006 Phosphatidylinositol 3-phosphate recognition and membrane docking by the FYVE domain. *Biochimica et Biophysica Acta* **1761** 868–877.
- Le Blanc C, Mironneau C, Barbot C, Henaff M, Bondeva T, Wetzker R & Macrez N 2004 Regulation of vascular L-type Ca^{2+} channels by phosphatidylinositol 3,4,5-trisphosphate. *Circulation Research* **95** 300–307.
- Lemmon MA 2007 Pleckstrin homology (PH) domains and phosphoinositides. *Biochemical Society Symposium* **74** 81–93.
- Lemmon MA, Ferguson KM, O'Brien R, Sigler PB & Schlessinger J 1995 Specific and high-affinity binding of inositol phosphates to an isolated pleckstrin homology domain. *PNAS* **92** 10472–10476.
- Liang J & Slingerland JM 2003 Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell Cycle* **2** 339–345.
- Lindmo K & Stenmark H 2006 Regulation of membrane traffic by phosphoinositide 3-kinases. *Journal of Cell Science* **119** 605–614.
- Lindvall J & Islam TC 2002 Interaction of Btk and Akt in B cell signaling. *Biochemical and Biophysical Research Communications* **293** 1319–1326.
- Macrez N, Mironneau C, Carricaburu V, Quignard JF, Babich A, Czupalla C, Nurnberg B & Mironneau J 2001 Phosphoinositide 3-kinase isoforms selectively couple receptors to vascular L-type Ca^{2+} channels. *Circulation Research* **89** 692–699.
- Maffucci T & Falasca M 2007 Phosphoinositide 3-kinase-dependent regulation of phospholipase Cgamma. *Biochemical Society Transactions* **35** 229–230.
- Maffucci T, Brancaccio A, Piccolo E, Stein RC & Falasca M 2003 Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. *EMBO Journal* **22** 4178–4189.
- Mansour E, Pereira FG, Araujo EP, Amaral ME, Morari J, Ferraroni NR, Ferreira DS, Lorand-Metze I & Velloso LA 2006 Leptin inhibits apoptosis in thymus through a janus kinase-2-independent, insulin receptor substrate-1/phosphatidylinositol-3 kinase-dependent pathway. *Endocrinology* **147** 5470–5479.
- Michell RH 2007 Evolution of the diverse biological roles of inositols. *Biochemical Society Symposium* **74** 223–246.
- Migliaccio A, Di Domenico M, Castoria G, de Falco A, Bontempo P, Nola E & Auricchio F 1996 Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. *EMBO Journal* **15** 1292–1300.
- Munzberg H & Myers MG Jr 2005 Molecular and anatomical determinants of central leptin resistance. *Nature Neuroscience* **8** 566–570.
- Murga C, Fukuhara S & Gutkind JS 2000 A novel role for phosphatidylinositol 3-kinase beta in signaling from G protein-coupled receptors to Akt. *Journal of Biological Chemistry* **275** 12069–12073.
- Naga Prasad SV, Esposito G, Mao L, Koch WJ & Rockman HA 2000 Gbetagamma-dependent phosphoinositide 3-kinase activation in hearts with *in vivo* pressure overload hypertrophy. *Journal of Biological Chemistry* **275** 4693–4698.
- Nakae J, Kitamura T, Ogawa W, Kasuga M & Accili D 2001 Insulin regulation of gene expression through the forkhead transcription factor Foxo1 (Fkhr) requires kinases distinct from Akt. *Biochemistry* **40** 11768–11776.
- Pacold ME, Suire S, Perisic O, Lara-Gonzalez S, Davis CT, Walker EH, Hawkins PT, 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.
- Patrucco E, Notte A, Barberis L, Selvetella G, Maffei A, Brancaccio M, Marengo S, Russo G, Azzolino O, Rybalkin SD *et al.* 2004 PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* **118** 375–387.
- Patten RD, Pourati I, Aronovitz MJ, Baur J, Celestin F, Chen X, Michael A, Haq S, Nuedling S, Grohe C *et al.* 2004 17beta-estradiol reduces cardiomyocyte apoptosis *in vivo* and *in vitro* via activation of phosphoinositide-3 kinase/Akt signaling. *Circulation Research* **95** 692–699.
- Pedram A, Razandi M, Aitkenhead M & Levin ER 2005 Estrogen inhibits cardiomyocyte hypertrophy *in vitro*. Antagonism of calcineurin-related hypertrophy through induction of MCP1. *Journal of Biological Chemistry* **280** 26339–26348.
- Plum L, Schubert M & Bruning JC 2005 The role of insulin receptor signaling in the brain. *Trends in Endocrinology and Metabolism* **16** 59–65.
- Pons S, Asano T, Glasheen E, Miralpeix M, Zhang Y, Fisher TL, Myers MG Jr, Sun XJ & White MF 1995 The structure and function of p55PIK reveal a new regulatory subunit for phosphatidylinositol 3-kinase. *Molecular and Cellular Biology* **15** 4453–4465.
- Quignard JF, Mironneau J, Carricaburu V, Fournier B, Babich A, Nurnberg B, Mironneau C & Macrez N 2001 Phosphoinositide 3-kinase gamma mediates angiotensin II-induced stimulation of L-type calcium channels in vascular myocytes. *Journal of Biological Chemistry* **276** 32545–32551.
- Ramm G, Laranca M, Guilhaus M & James DE 2006 A role for 14-3-3 in insulin-stimulated GLUT4 translocation through its interaction with the RabGAP AS160. *Journal of Biological Chemistry* **281** 29174–29180.
- Ren SY, Bolton E, Mohi MG, Morrione A, Neel BG & Skorski T 2005 Phosphatidylinositol 3-kinase p85{alpha} subunit-dependent interaction with BCR/ABL-related fusion tyrosine kinases: molecular mechanisms and biological consequences. *Molecular and Cellular Biology* **25** 8001–8008.
- Rodriguez A, Fortuno A, Gomez-Ambrosi J, Zalba G, Diez J & Fruhbeck G 2007 The inhibitory effect of leptin on angiotensin II-induced vasoconstriction in vascular smooth muscle cells is mediated via a nitric oxide-dependent mechanism. *Endocrinology* **148** 324–331.

- Rondinone CM, Wang LM, Lonnroth P, Wesslau C, Pierce JH & Smith U 1997 Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *PNAS* **94** 4171–4175.
- Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J *et al.* 2005 PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Research* **65** 2554–2559.
- Sabatini DM 2006 mTOR and cancer: insights into a complex relationship. *Nature Reviews. Cancer* **6** 729–734.
- Saward L & Zahradka P 1997 Angiotensin II activates phosphatidylinositol 3-kinase in vascular smooth muscle cells. *Circulation Research* **81** 249–257.
- Schmidt M, Fernandez de Mattos S, van der Horst A, Klompaker R, Kops GJ, Lam EW, Burgering BM & Medema RH 2002 Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. *Molecular and Cellular Biology* **22** 7842–7852.
- Schreyer S, Ledwig D, Rakatzi I, Kloting I & Eckel J 2003 Insulin receptor substrate-4 is expressed in muscle tissue without acting as a substrate for the insulin receptor. *Endocrinology* **144** 1211–1218.
- Sciacchitano S & Taylor SI 1997 Cloning, tissue expression, and chromosomal localization of the mouse *IRS-3* gene. *Endocrinology* **138** 4931–4940.
- Shepherd PR 2005 Mechanisms regulating phosphoinositide 3-kinase signalling in insulin-sensitive tissues. *Acta Physiologica Scandinavica* **183** 3–12.
- Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW & Liao JK 2000 Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature* **407** 538–541.
- Sonenberg N & Pause A 2006 Signal transduction. Protein synthesis and oncogenesis meet again. *Science* **314** 428–429.
- Stephens LR, Eguinoa A, Erdjument-Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P *et al.* 1997 The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* **89** 105–114.
- Stoica GE, Franke TF, Wellstein A, Czubyk F, List HJ, Reiter R, Morgan E, Martin MB & Stoica A 2003 Estradiol rapidly activates Akt via the ErbB2 signaling pathway. *Molecular Endocrinology* **17** 818–830.
- Suire S, Hawkins P & Stephens L 2002 Activation of phosphoinositide 3-kinase gamma by Ras. *Current Biology* **12** 1068–1075.
- Suire S, Coadwell J, Ferguson GJ, Davidson K, Hawkins P & Stephens L 2005 p84, a new Gbetagamma-activated regulatory subunit of the type IB phosphoinositide 3-kinase p110gamma. *Current Biology* **15** 566–570.
- Suire S, Condliffe AM, Ferguson GJ, Ellison CD, Guillou H, Davidson K, Welch H, Coadwell J, Turner M, Chilvers ER *et al.* 2006 Gbetagammass and the Ras binding domain of p110gamma are both important regulators of PI(3)Kgamma signalling in neutrophils. *Nature Cell Biology* **8** 1303–1309.
- Sun XJ, Pons S, Wang LM, Zhang Y, Yenush L, Burks D, Myers MG Jr, Glasheen E, Copeland NG, Jenkins NA *et al.* 1997 The *IRS-2* gene on murine chromosome 8 encodes a unique signaling adapter for insulin and cytokine action. *Molecular Endocrinology* **11** 251–262.
- Sun M, Paciga JE, Feldman RI, Yuan Z, Coppola D, Lu YY, Shelley SA, Nicosia SV & Cheng JQ 2001 Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. *Cancer Research* **61** 5985–5991.
- Szanto I & Kahn CR 2000 Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. *PNAS* **97** 2355–2360.
- Takahashi T, Taniguchi T, Konishi H, Kikkawa U, Ishikawa Y & Yokoyama M 1999 Activation of Akt/protein kinase B after stimulation with angiotensin II in vascular smooth muscle cells. *American Journal of Physiology* **276** H1927–H1934.
- Taniguchi CM, Tran TT, Kondo T, Luo J, Ueki K, Cantley LC & Kahn CR 2006 Phosphoinositide 3-kinase regulatory subunit p85alpha suppresses insulin action via positive regulation of PTEN. *PNAS* **103** 12093–12097.
- Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A, Inukai K, Asano T, Kaburagi Y, Ueki K *et al.* 1999 Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nature Genetics* **21** 230–235.
- Thong FS, Dugani CB & Klip A 2005 Turning signals on and off: GLUT4 traffic in the insulin-signaling highway. *Physiology* **20** 271–284.
- Trotman LC, Alimonti A, Scaglioni PP, Koutcher JA, Cordon-Cardo C & Pandolfi PP 2006 Identification of a tumour suppressor network opposing nuclear Akt function. *Nature* **441** 523–527.
- Tschopp O, Yang ZZ, Brodbeck D, Dummmler BA, Hemmings-Mieszczak M, Watanabe T, Michaelis T, Frahm J & Hemmings BA 2005 Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. *Development* **132** 2943–2954.
- Ueki K, Fruman DA, Brachmann SM, Tseng YH, Cantley LC & Kahn CR 2002a Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. *Molecular and Cellular Biology* **22** 965–977.
- Ueki K, Yballe CM, Brachmann SM, Vicent D, Watt JM, Kahn CR & Cantley LC 2002b Increased insulin sensitivity in mice lacking p85beta subunit of phosphoinositide 3-kinase. *PNAS* **99** 419–424.
- Ueki K, Fruman DA, Yballe CM, Fasshauer M, Klein J, Asano T, Cantley LC & Kahn CR 2003 Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3-kinase in insulin signaling. *Journal of Biological Chemistry* **278** 48453–48466.
- Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegri PR, Kozma SC, Auwerx J *et al.* 2004 Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* **431** 200–205.
- Vanhaesebroeck B & Alessi DR 2000 The PI3K-PDK1 connection: more than just a road to PKB. *Biochemical Journal* **346** 561–576.
- Vanhaesebroeck B, Leever SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ & Waterfield MD 2001 Synthesis and function of 3-phosphorylated inositol lipids. *Annual Review of Biochemistry* **70** 535–602.
- Vecchione C, Patrucco E, Marino G, Barberis L, Poulet R, Aretini A, Maffei A, Gentile MT, Storto M, Azzolino O *et al.* 2005 Protection from angiotensin II-mediated vasculotoxic and hypertensive response in mice lacking PI3Kgamma. *Journal of Experimental Medicine* **201** 1217–1228.
- Viard P, Butcher AJ, Halet G, Davies A, Nurnberg B, Hebllich F & Dolphin AC 2004 PI3K promotes voltage-dependent calcium channel trafficking to the plasma membrane. *Nature Neuroscience* **7** 939–946.
- Voigt P, Brock C, Nurnberg B & Schaefer M 2005 Assigning functional domains within the p101 regulatory subunit of phosphoinositide 3-kinase gamma. *Journal of Biological Chemistry* **280** 5121–5127.
- Walker EH, Perisic O, Ried C, Stephens L & Williams RL 1999 Structural insights into phosphoinositide 3-kinase catalysis and signalling. *Nature* **402** 313–320.
- Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP & Williams RL 2000 Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Molecular Cell* **6** 909–919.
- Wang Y, Kuropatwinski KK, White DW, Hawley TS, Hawley RG, Tartaglia LA & Baumann H 1997 Leptin receptor action in hepatic cells. *Journal of Biological Chemistry* **272** 16216–16223.
- Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI *et al.* 1998 Disruption of *IRS-2* causes type 2 diabetes in mice. *Nature* **391** 900–904.
- Wulfschleger S, Loewith R & Hall MN 2006 TOR signaling in growth and metabolism. *Cell* **124** 471–484.
- Xu Y, Seet LF, Hanson B & Hong W 2001 The Phox homology (PX) domain, a new player in phosphoinositide signalling. *Biochemical Journal* **360** 513–530.
- Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA & Backer JM 1998 Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. *Molecular and Cellular Biology* **18** 1379–1387.
- Zhao AZ, Shinohara MM, Huang D, Shimizu M, Eldar-Finkelman H, Krebs EG, Beavo JA & Bornfeldt KE 2000 Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. *Journal of Biological Chemistry* **275** 11348–11354.

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