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REVIEW ARTICLE

Multiple roles for the p85α isoform in the regulation and function of PI3K signalling and receptor trafficking

Paul MELLOR*†, Levi A. FURBER*, Jennifer N. K. NYARKO* and Deborah H. ANDERSON*†[1]

*Department of Biochemistry, University of Saskatchewan, Saskatoon, SK S7N 5E5 Canada, and †Cancer Research Unit, Research Division, Saskatchewan Cancer Agency, Saskatoon, SK S7N 4H4, Canada

The p85α protein is best known as the regulatory subunit of class 1A PI3Ks (phosphoinositide 3-kinases) through its interaction, stabilization and repression of p110-PI3K catalytic subunits. PI3Ks play multiple roles in the regulation of cell survival, signalling, proliferation, migration and vesicle trafficking. The present review will focus on p85α, with special emphasis on its important roles in the regulation of PTEN (phosphatase and tensin homologue deleted on chromosome 10) and Rab5 functions. The phosphatidylinositol-3-phosphatase PTEN directly counteracts PI3K signalling through dephosphorylation of PI3K lipid products. Thus the balance of p85α–p110 and p85α–PTEN complexes determines the signalling output of the PI3K/PTEN pathway, and under conditions of reduced p85α levels, the p85α–PTEN complex is selectively reduced, promoting PI3K signalling. Rab5 GTPases are important during the endocytosis, intracellular trafficking and degradation of activated receptor complexes. The p85α protein helps switch off Rab5, and if defective in this p85α function, results in sustained activated receptor tyrosine kinase signalling and cell transformation through disrupted receptor trafficking. The central role for p85α in the regulation of PTEN and Rab5 has widened the scope of p85α functions to include integration of PI3K activation (p110-mediated), deactivation (PTEN-mediated) and receptor trafficking/signalling (Rab5-mediated) functions, all with key roles in maintaining cellular homoeostasis.

Key words: actin reorganization, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), Rab5 GTPase, receptor degradation, receptor endocytosis, vesicle trafficking.

INTRODUCTION

Class 1A PI3Ks (phosphoinositide 3-kinases) consist of a p85 regulatory subunit and a p110 catalytic subunit. The p85 isoforms are a group of related proteins encoded by the *Pik3r1* (p85α, p55α, p50α), *Pik3r2* (p85β) and *Pik3r3* (p55γ) genes. The smaller 50–55 kDa isoforms contain two SH2 (Src homology 2) domains [nSH2 (N-terminal SH2 domain) and cSH2 (C-terminal SH2 domain)] and a p110-binding domain [iSH2 (inter SH2 domain)] (Figure 1). The larger p85 kDa isoforms contain the domains within the small isoforms, but also have an N-terminal SH3 domain and a GAP (GTPase-activating protein) domain [also called a BH (BCR homology) domain], as well as two proline-rich regions, to provide added binding functions and regulatory capacities. By far the most is known about the p85α isoform and is the major focus of the present review. For information about the other isoforms, please refer to previous reviews [1,2].

The p85α protein is best known as the regulatory subunit of PI3K. As such, it has major well-described functions in the stabilization and regulation of the p110 catalytic subunit of PI3K [3]. PI3K is activated by many RTKs (receptor tyrosine kinases) {e.g. PDGFR [PDGF (platelet-derived growth factor) receptor], EGFR [EGF (epidermal growth factor) receptor] and IR (insulin receptor)} or cytoplasmic tyrosine kinases (e.g. Abl, Src-family, Jak and ZAP70), some of which associate with, or are activated by, receptors lacking intrinsic tyrosine kinase activity (e.g. cytokine receptors, B-cell or T-cell receptors and integrins) (reviewed in [4,5]). In quiescent cells, a pre-existing p85–p110 complex is cytosolic and p110-encoded PI3K activity is repressed. In response to upstream activating signals, typically the activation of tyrosine kinase activity at the plasma membrane, new tyrosine phosphorylation sites are generated on receptors that create binding sites for the SH2 domains within p85. In addition to directly binding RTKs, p85 can indirectly bind to receptors through adaptor proteins such as the IR substrates IRS-1 and IRS-2. Following activation of the IR or IGF-1 (insulin-like growth factor 1) receptor, IRS-1 or IRS-2 is recruited to the receptor through a phosphotyrosine-binding domain [6]. The C-termini of IRS-1 and IRS-2 contain tyrosine phosphorylation sites that enable the binding and activation of PI3K through SH2 domains in p85 [7]. The binding of p85 SH2 domains to these tyrosine phosphorylation sites relieves the repression on p110 catalytic activity [8]. In addition, this interaction recruits the p85–p110 complex to the plasma membrane, the location of its lipid substrate PtdIns(4,5)P_2. Phosphorylation on the 3-position of the inositol ring results in the generation of PtdIns(3,4,5)P_3. PI3K can also be activated by association with the activated Ras GTPase at the plasma membrane, via a direct interaction between

Abbreviations used: ABD, adaptor-binding domain; BH, BCR homology; cSH2, C-terminal Src homology 2 domain; EEA1, early endosomal antigen 1; EGF, epidermal growth factor; EGFR, EGF receptor; GAP, GTPase-activating protein; GDI, guanine-nucleotide-dissociation inhibitor; GDF, GDI displacement factor; GEF, guanine-nucleotide-exchange factor; GPCR, G-protein-coupled receptor; IL, interleukin; IR, insulin receptor; IRS, IR substrate; iSH2, inter Src homology 2 domain; NSF, *N*-ethylmaleimide-sensitive fusion protein; nSH2, N-terminal Src homology 2 domain; PAC, PTEN-associated complex; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PDK1, 3-phosphoinositide-dependent protein kinase 1; PI, phosphatidylinositol; PI3K, phosphoinositide 3-kinase; PIN, prostatic intraepithelial neoplasia; PI3-Pase, PI-3-phosphatase; PI4-Pase, PI-4-phosphatase; PI5-Pase, PI-5-phosphatase; PKA, protein kinase A; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RTK, receptor tyrosine kinase; SH, Src homology; SNARE, soluble NSF-attachment protein receptor; XBP-1, X-box-binding protein-1.

[1] To whom correspondence should be addressed (email deborah.anderson@saskcancer.ca).

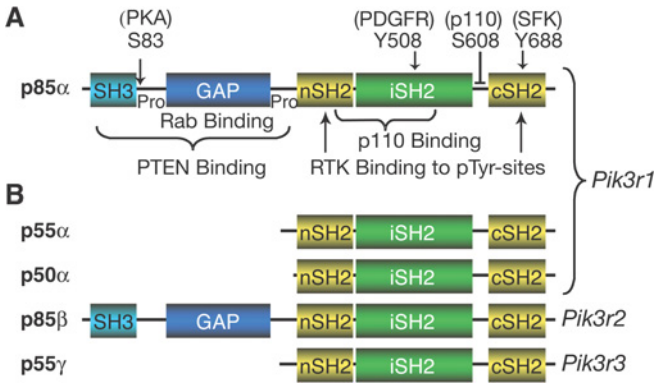

Figure 1 Domain structures of p85 isoforms, and regulatory phosphorylation sites for p85α

Pik3r1 encodes p85α, p55α and p50α. *Pik3r2* encodes p85β, whereas *Pik3r3* encodes p55γ. Common to all isoforms are the presence of two C-terminal SH2 domains flanking a p110-binding domain (iSH2). The smaller isoforms differ at their N-terminus and contain a unique 35-amino-acid (p55α) or five-amino-acid (p50α, p50γ) sequence. Unique to the larger isoforms are the N-terminal SH3 domain, GAP domain (also called BH domain) and two proline-rich regions. (**A**) p85α domains, showing selected binding proteins and phosphorylation sites. Phosphorylations with positive effects on PI3K activity are shown with arrows, whereas those with negative effects on PI3K activity are shown with bars. (**B**) Domains present in other related isoforms.

Ras-GTP and p110 [9–11]. The PI3-Pase [PI (phosphatidylinositol)-3-phosphatase] PTEN (phosphatase and tensin homologue deleted on chromosome 10) regulates the strength and duration of PI3K signalling by dephosphorylating PtdIns(3,4,5)P_3 back to PtdIns(4,5)P_2, the regulation of which is discussed in greater detail below.

PtdIns(3,4,5)P_3 lipids are important lipid messengers that relocalize a number of downstream signalling proteins including PDK1 (3-phosphoinositide-dependent protein kinase 1) and Akt [also known as PKB (protein kinase B)], resulting in their activation [5,12]. These critical signalling proteins activate many pathways leading to increased cell growth, cell-cycle progression, cell migration and cell survival through the activation of numerous target proteins [13,14]. Several reviews have described these pathways in detail [15–18].

PI3K lipid products also interact with cytoskeletal-associated proteins including the Rac GTPase, a member of the Rac/Rho/Cdc42-GTPase family [19], which have important roles in reorganization of the actin cytoskeleton and actin regulatory proteins (e.g. α-actinin, profilin and gelsolin) [1,12]. Actin, dynamin [20,21] and other plasma-membrane molecules play major roles in the formation of endosomes during the internalization and trafficking of cell-surface receptors [22,23] and/or during virus entry [24,25].

The p85α protein has additional functions besides the regulation of p110-PI3K activity, and the combination of these functions may serve to provide an organized, integrated and sequential mechanism to regulate key cellular processes. Since it was first cloned 20 years ago, p85α has been shown to bind to many other partners in addition to p110 and tyrosine-phosphorylated proteins. The p85α protein is also phosphorylated and ubiquitinated, modifications that can have an impact on its regulation. In the present review, we will summarize these p85α (referred to as p85) modifications and many of its binding partners, with emphasis on those that bind to the N-terminal half of p85, including its SH3 and GAP domains. We will also highlight some of these p85-binding partners, in particular the PTEN lipid phosphatase that counteracts PI3K signalling and the Rab5 GTPase that has a major role in receptor trafficking and down-regulation.

POST-TRANSLATIONAL MODIFICATIONS OF p85

Post-translational modifications of p85 are shown in Figure 1(A). The p85 protein can be phosphorylated on several tyrosine and serine sites, some of which are known to influence its function. The p110 protein has protein kinase activity in addition to its lipid kinase activity [26]. It phosphorylates p85 on Ser^{608} decreasing p85–p110-PI3K activity by 80 %, suggesting that it may be a negative-feedback mechanism to ensure that PI3K activation is transient [26]. In 3T3-L1 adipocytes, protein phosphatase 2C dephosphorylates p85 and activates PI3K activity, perhaps by dephosphorylation of this negative regulatory Ser^{608} site on p85 [27].

PKA (protein kinase A) phosphorylates p85 on Ser^{83}, enhances Ras binding of PI3K and results in PI3K activation [28,29]. The phosphorylated p85 protein also binds the regulatory subunit of PKA and recruits PKAII to the membrane, suggesting a mutual regulation that could help co-ordinate cell signalling responses.

The p85 protein can also be phosphorylated on several tyrosine residues. Abl and Src-family tyrosine kinases phosphorylate p85 on Tyr^{688} [30,31]. This has been suggested to alter the SH2-binding properties of p85 [30], and relieve the inhibition of p85 on p110-PI3K [31], resulting in PI3K activation. Phosphorylation of p85 on Tyr^{688} has also been proposed to promote some *de novo* p85–p110 heterodimer formation [32]. p85 is also phosphorylated on Tyr^{508} by the PDGFR [33] and in response to IL (interleukin)-8 and/or GM-CSF (granulocyte/macrophage colony-stimulating factor) [34]. The significance of Tyr^{508} phosphorylation of p85 is not clear, since mutation of this site appears to have only a minor effect on PI3K activity in response to PDGF [33]. Both SHP-1 (SH2 domain-containing protein tyrosine phosphatase 1) and CD148 are tyrosine phosphatases that dephosphorylate p85 [31,35].

In addition to phosphorylation, the p85 protein can be ubiquitinated by Cbl-b, a modification that does not induce degradation [36], but prevents recruitment of p85 to CD28, a co-receptor involved in T-cell receptor signalling [37]. In the absence of Cbl-b, p85–p110 produces PI3K lipid products that bind the PH domain (pleckstrin homology domain) of Vav, induce Vav phosphorylation and activate its nucleotide exchange towards Rac [38,39]. The lack of Cbl-b uncouples T-cell receptor signalling from the usual co-stimulatory CD28 responses causing proliferation and IL-2 production that results in autoimmune diseases. The net effect is that p85 ubiquitination by Cbl-b blocks PI3K activity by preventing its recruitment to CD28/T-cell receptor [37] to effect Vav activation indirectly.

p85 BINDING PARTNERS

A list of p85α domains and their binding partners are shown in Table 1.

The iSH2 domain of p85 is the major site of interaction with the p110 protein, although the nSH2 domain of p85 also has important contacts with p110 [8,40]. Further detailed discussions of the p85–p110 interaction, cancer-associated mutations that effect the complex and the regulation of PI3K activity are the focus of a later section within the present review. The iSH2 domain of p85 also binds α/β- and γ-tubulins, proteins that function in microtubule-dependent vesicle trafficking (α/β-tubulin) and the microtubule-organizing centre within centrosomes (γ-tubulin) [41]. The role of this interaction in microtubule formation or stability is not clear, but it has been suggested to regulate budding or fusion of vesicles associated with microtubules [41].

Table 1 p85α domains and their binding partners

p85 domain	Binding partner	Function	Reference(s)
N-terminal half			
SH3 + proline-rich + GAP	p85	Intramolecular homodimerization	[53]
SH3	FAK	PI3K activation in integrin signalling	[63]
	CAS	PI3K activation and cell migration	[64]
	Apoptin	PI3K activation and tumour cell apoptosis	[65]
	Ruk	PI3K inhibition and neuronal apoptosis	[66]
	SNX9	CD28 receptor endocytosis and actin reorganization	[67]
	Dynamin	Endocytosis and vesicle scission	[69,70]
	Cbl	Protein localization, ubiquitination and degradation	[71]
	Bcr-Abl	Bcr-Abl-mediated transformation	[68]
Proline-rich	Grb2	PI3K activation	[73,74]
	Crk	PI3K activation	[75]
	α-Actinin	Actin reorganization	[76]
	Abl, Src-family	PI3K activation	[77–80]
GAP (BH)	XBP-1	Nuclear translocation	[61]
	Rac, Cdc42	PI3K activation and actin reorganization	[82,83]
	Rab5	RTK and β-integrin trafficking	[48,84,89,198]
	PTEN	PI3-Pase activation	[93]
C-terminal half			
nSH2 + iSH2	p110	PI3K activity, stability and localization	[8,40]
iSH2	Tubulins	Not clear; vesicle trafficking?	[41]
Phospho-tyrosine-dependent			
SH2s	RTKs	PI3K activation in growth factor signalling	Reviewed in [4,5]
	Adapters (e.g. IRS-1/2, Grb2, Gab1/2, Shc, Crk)	PI3K activation in insulin, B-cell, T-cell and cytokine signalling	Reviewed in [4,5]
	Caspase 8	β-Integrin trafficking	[48]
	β-Catenin	PI3K activation in cell adhesion	[47]
Phospho-tyrosine-independent			
SH2s	A-Raf	PI3K inhibition	[49–51]
	Ankyrin3	Receptor trafficking and lysosomal degradation	[52]

The SH2 domains of p85 bind to pYxxM motifs (pY is phosphorylated tyrosine) on many proteins, but in particular activated RTKs (e.g. PDGFR and EGFR) and adapter proteins (e.g. IRS-1 [42], Grb2, Gab1/2 [43], Shc [44,45], Crk-L [46] and β-catenin [47]). As noted above, these phosphotyrosine-dependent p85 SH2 interactions serve two functions: they de-repress the associated p110-PI3K activity and relocalize p85–p110 to the plasma membrane, the location of its lipid substrates. There is also at least one example of a p85 SH2 domain interaction with tyrosine-phosphorylated caspase 8 that serves to sequester p85 to prevent its Rab5 regulatory functions [48], discussed further in a later section of the present review.

In addition, p85 SH2 domains can form rare phosphotyrosine-independent interactions with target proteins, including the A-Raf serine/threonine kinase [49–51] and the smaller isoforms of ankyrin3 [52]. A-Raf activation down-regulates PI3K activity, whereas the small isoforms of ankyrin3 play a role in receptor trafficking and degradation.

SH3 domains typically bind to proline-rich target sequences, usually containing a PxxP motif. The p85 protein can form homodimers through interactions between the SH3 domain of one p85 with the first proline-rich region of a second p85 protein [53]. These interactions are not observed for p85 proteins already associated with p110 in a p85–p110 complex. Since p85 expressed in excess of p110 has a shorter half-life than p110-associated p85 [54], association with p110 may serve to stabilize p85. PI3K isoform expression has been reviewed in detail previously [2]. Some studies suggest that, at least in some cell types, p85 proteins are completely bound with p110 [55], but there are some examples of p85 expression in excess of p110. For example, in skeletal muscle of obese and Type 2 diabetic mice [56], as well as in response to growth hormone [57–59], p85 protein expression, but not p110 expression, is substantially increased, suggesting that surplus p85, not in a p85–p110 complex, is sometimes present. The observed increased p85 expression induced by placental growth hormone has been suggested to produce insulin resistance associated with physiological adaptations during normal pregnancy, required to restrict maternal glucose uptake and ensure that nutrients are available for the developing foetus [58].

It has been suggested that if p85 proteins are not already associated with p110 in p85–p110 complexes they would probably form p85–p85 homodimers, an association also mediated in part by weak hydrophobic GAP–GAP domain interactions [53,60]. One interesting possibility is that once p110 is completely complexed with p85, an absolute requirement for p110 stability, any surplus p85 forms p85–p85 homodimers via intramolecular SH3–proline-rich and/or GAP–GAP interactions. Perhaps in response to a stimulus, the p85–p85 homodimers would dissociate in favour of alternative interactions with binding partners that interact with the newly available p85 SH3, proline-rich and/or GAP domains. A recent report has shown that ectopically expressed p85α and p85β can be co-immunoprecipitated from cells, and that the amount of this complex decreased upon insulin stimulation [61]. These results suggest that a p85α–p85β dimer can form in non-stimulated cells and that it is disrupted upon stimulation (insulin in this example). The authors of that study [61] further showed that p85α and p85β each associated with XBP-1 (X-box-binding protein-1) upon insulin stimulation with no p110 observed in these complexes, an interaction important for XBP-1 nuclear translocation and mediated by the GAP domain of p85. This suggests that the function of p85–p85 homodimerization may be to protect or mask SH3, proline-rich and GAP domain binding surfaces within p85 until the dimer is disrupted in favour of new interactions with other binding partners. In support of such a mechanism, p85 SH3 interactions with p85 proline-rich regions are weaker

than interactions with other target proline-rich sequences [62].

The SH3 domain of p85 also binds proline-rich regions of many target proteins (see Table 1), and in several instances these interactions serve to regulate p85–p110-PI3K activity [63–71]. Pre-formed complexes between p85 SH3 and Cbl proline-rich regions have been suggested as a method to hold proteins in close proximity with each other. This may be a mechanism to facilitate the subsequent p85–p110-PI3K activation by p85 SH2-domain-mediated interactions with tyrosine-phosphorylated Cbl upon Lyn kinase activation [71]. The functions of other p85 SH3 complexes include: (i) providing new mechanisms to activate PI3K, independent of SH2 domain binding [63–65], (ii) inhibiting PI3K [66] and (iii) coupling CD28 receptor endocytosis with actin polymerization [67,72].

The proline-rich regions of p85 bind to several target proteins via interactions with their SH3 domains, including adapter proteins Grb2 [73,74] and Crk [75] that link p85–p110-PI3K to upstream activating signals. The proline-rich regions of p85 also bind to the SH3 domain of α-actinin, an actin-binding protein, in a PtdIns(4,5)P_2-dependent manner (thought to cause a conformational change in α-actinin), as a mechanism to regulate cytoskeletal reorganization [76]. One major group of p85 proline-rich-binding target proteins is the cytoplasmic tyrosine kinases, including Abl and the Src-family kinases [77–80]. These are phosphorylation-independent interactions that mediate binding and activation of PI3K activity.

The GAP (also called BH) domain of p85 was initially identified through sequence homology as a probable GAP domain for the Rac/Rho/Cdc42 family of GTPases. These G-proteins regulate actin dynamics important for many cell functions, including cell shape, migration, cytokinesis and vesicle trafficking [81]. GAP proteins stimulate the intrinsic GTP hydrolysis activity of G-proteins to help switch them from an active GTP-bound conformation to an inactive GDP-bound state. At nanomolar concentrations, p85 binds to the active GTP-forms of Rac1 and Cdc42 (not RhoA), resulting in PI3K activation, but at these low concentrations p85 does not exert GAP activity towards these G-proteins [82,83]. At micromolar concentrations, p85 has GAP activity towards Rac1 and Cdc42 [84], but it is not known whether these high localized protein concentrations are achieved in cells.

GAP proteins typically contribute a catalytic arginine residue (termed an arginine finger) to stabilize the transition state during GTP hydrolysis, and also stabilize the switch I and II regions within the G-protein that take on different conformations in the GTP-bound compared with GDP-bound states. A sequence alignment between different GAP proteins was used to compare those with high GAP activity towards Cdc42 (e.g. Cdc42GAP) and those with low GAP activity towards Cdc42 (e.g. p85) [85]. This analysis indicated that the arginine finger within Cdc42GAP (Arg^{305}) is conserved within p85 (Arg^{151}), although several of the switch stabilization residues in Cdc42GAP were not conserved in p85 [85]. This has been suggested as the reason why p85 is a poor GAP towards Cdc42, and may instead function primarily as a Cdc42-GTP (and Rac1-GTP)-binding protein [82,86–88].

More recently, our laboratory has shown that p85 has GAP activity towards Rab5 and Rab4 [84,89], GTPases important for the regulation of RTK trafficking [90–92]. This catalytic function for p85 was only weakly affected by mutation of Arg^{151}, the predicted arginine finger residue. Instead p85 GAP activity was severely impaired by a single point mutation (R274A), located elsewhere within the predicted G-protein-binding site of the p85 GAP domain [60]. Thus Arg^{274} is the critical arginine finger residue within p85. A detailed discussion of this new function for p85 as a Rab GAP to regulate receptor trafficking follows below in a later section.

Recently, our group has shown that the N-terminal part of p85, including its GAP domain, binds directly to and positively regulates PTEN, the lipid phosphatase that counteracts PI3K activity (PI3-Pase) [93]. This dual function for p85 as a regulator of p110-PI3K and PTEN-PI3Pase activities provides a direct link between the enzymes that control the level of cellular PtdIns(3,4,5)P_3 lipids, and thus the magnitude and duration of PI3K signalling [94]. A detailed description of these results and their implications is the focus of a subsequent section of the present review.

PI3Ks AND CLASS 1A p85-MEDIATED REGULATION OF p110-PI3K

PI3Ks phosphorylate PI lipids at the 3-position and are classified according to how they are activated, the structure of their heterodimer and their substrate specificity [95]. The most commonly studied are class I PI3Ks that generate PtdIns(3,4,5)P_3, and which are further subdivided into class IA and class IB. Class IA PI3Ks are p85–p110 heterodimers. The five p85 regulatory subunits (p85α, p55α, p50α, p85β and p55γ) can form heterodimers with any of three p110 isoforms (p110α, p110β and p110δ). As noted above, class 1A heterodimers involving p110α are activated by tyrosine kinases. In contrast, heterodimers involving p110β can be activated by binding $G_{\beta/\gamma}$ subunits of heterotrimeric G-proteins in response to GPCR (G-protein-coupled receptor) activation, as well as by activated RTKs [96–98]. Expression of p110δ is predominantly restricted to the immune system in haemopoietic cells, whereas p110α and p110β are widely expressed in many cell types [99]. The class IB PI3Ks are activated by GPCRs and consist of a regulatory subunit (p101, p84 or p87PIKAP) associated with the p110γ catalytic subunit [100].

In contrast, the class II PI3Ks consist of three catalytic subunits, C2α, C2β and C2γ, but have no regulatory proteins. This class of PI3K utilizes PI and PtdIns4*P* as substrates to generate PtdIns3*P* and PtdIns(3,4)P_2 [101–103]. Class III PI3Ks exist as multimeric complexes containing the Vps34 catalytic subunit and the p150 regulatory subunit [104]. The class III PI3Ks can only utilize PI as a substrate, generating PtdIns3*P* (reviewed in [4]).

Class IA p110 proteins consist of five domains: an ABD (adaptor-binding domain; i.e. the main p85-binding domain), a Ras-binding domain, a C2 domain, a helical domain and a catalytic domain [105]. The interaction between the p110α and p85α protein is mediated primarily by extensive contacts between the ABD domain of p110α and the iSH2 domain of p85. The C2 domain of p110α (e.g. residues Asn^{345} and Glu^{453}) also interacts with the iSH2 domain of p85 (e.g. residues Asp^{560} and Asn^{564}) [105]. A third site of interaction between p110α and p85α provides insight into the mechanism of PI3K activity repression/de-repression. The p110α helical domain (residues Glu^{542} and Glu^{545}) contacts the p85 nSH2 domain (residues Lys^{379} and Arg^{340}), and inhibits p110α catalytic activity [8,40]. The p85 nSH2 domain cannot bind to the p110α helical domain and phosphopeptide (that mimics the activated receptor) at the same time. This suggests a mechanism for p110-PI3K activation as a result of p85 nSH2 domain binding to upstream tyrosine-phosphorylated receptors and/or adapter proteins that displace the inhibitory p110α helical domain interaction [105]. In the case of p110β, an additional unique interaction between the cSH2 domain of p85 and the kinase domain of p110β is also required for inhibition [106].

The activated GTP-bound forms of Ras interact with several PI3K class I isoforms (reviewed in [107]). Binding of Ras-GTP activates p85–p110α-PI3K activity [10], either via induction of

conformational changes in p110α, or by facilitating interactions with the plasma membrane [107]. The importance of Ras interactions for p110α-PI3K signalling was tested by knock-in expression of a p110α mutant defective for Ras binding [108]. Uncoupling p110α from Ras attenuated Akt activation induced by EGF and FGF (fibroblast growth factor), but not PDGF, suggesting a differential requirement for Ras in these signalling responses. In addition, knock-in expression of this Ras-binding-domain mutant of p110α in mice blocked oncogenic Ras-induced lung and skin tumorigenesis, suggesting that p110α has an important role downstream of Ras in oncogenesis [108].

In recent years, class IA PI3Ks have been shown to be frequently mutated in a number of cancer types (reviewed in [100,109–114]). A large number of mutations have been identified in the *Pik3ca* gene, encoding p110α, in a range of different types of cancer. Cells containing these mutations have activated p110α with increased Akt signalling, cell survival and tumorigenesis. These mutations are clustered (approximately 80 %) in hotspots in either the kinase (e.g. H1047R) or helical (e.g. E542K and E545K) domains, and are all oncogenic gain-of-function mutations [8,115–117]. The remaining 20 % of *Pik3ca* mutations are of low frequency and are found in every domain of p110α, with the exception of the Ras-binding domain. The lack of p110 mutations within the Ras-binding domain suggests that Ras associations are vital for tumorigenesis [108,118]. Considering the high frequency of p110α mutations in cancers, it is surprising that no oncogenic mutations have been identified in other p110 isoforms. However, these non-α isoforms are often overexpressed in tumours, and studies in chicken embryo fibroblasts have shown that overexpression is sufficient for oncogenic transformation [119–124].

The cancer-associated p110α H1047R mutation within the kinase domain results in catalytic activation that still requires p85 binding, but is independent of Ras [100,105]. More recently a structure–function analysis of the p110α H1047R mutant protein showed notable structural changes as a result of the mutation that increased its membrane interaction, as compared with wild-type p110α [125]. The PI3K activity of the p110α H1047R mutant was also differentially activated by distinct lipid membrane compositions much more significantly than the wild-type p110α. In contrast, the cancer-associated p110α E542K and E545K mutations within the helical domain result in p110α activation through disruption of inhibitory ionic interactions with basic residues within the p85 nSH2 domain (Lys^{379} and Arg^{340}, noted above) [105]. These helical domain p110α mutants still require binding to Ras [100].

Mutations in the *Pik3r1* gene (encoding p85α) are less common than *Pik3ca* (p110α) mutations and efforts have been made to determine the frequency and role these mutations may play in cancer [126,127]. Initially, the search for *Pik3r1* mutations in human cancers focused on the exons encoding the C-terminal portion of the p85α protein (nSH2, iSH2, cSH2) [128] and the ability of these mutations to release inhibitory effects on p110-encoded PI3K activity. Recent genome-wide screens for mutations in human tumour samples did not detect any *Pik3r1* mutations in tumours of the lung [129] and pancreas [130]. Other studies detected *Pik3r1* mutations in a few ovarian (3/80) [128], colon (1/60) [128], breast (1/62) [131] and pancreatic (1/6) [131] cancers. A higher frequency of *Pik3r1* mutations has been discovered in glioblastomas (7–10 %; 8/105 [126] and 9/91 [132]) and colorectal cancers (8 %; 9/108) [131].

The most common p85α mutation sites include those within the nSH2 domain (Arg^{348}, Gly^{376} and Leu^{380}) and iSH2 domain (Lys^{459}, Asp^{560}, Asn^{564} and Arg^{574}) [131]. They are gain-of-function mutations due to a loss of p110 inhibition and enhanced PI3K signalling, in a very similar manner to the mutations occurring in the C2 domain of p110α [115,116,131]. Asp^{560} and Asn^{564} make key contacts with the C2 domain of p110α, and disruption of this interaction through mutation would be functionally equivalent to mutation of Asn^{345} within the p110α C2 domain, the corresponding cancer-associated *Pik3ca* mutation [132]. The most oncogenic of the *Pik3r1* mutations studied were those that induced deletions in p85α, potentially disrupting the structure of the iSH2 domain, such as Asp^{459} + S460N or Asp^{560}–Asp^{565}, weakening interactions with the C2 domain of p110α [133,134]. Similarly, p85 mutations at residues N564D and D560Y or a two-amino-acid deletion (Asp^{579}–Asp^{580}) identified in colon cancer had no effect on the ability of p85 to bind and stabilize all isoforms of p110, but prevented p85α exerting its inhibitory effects on p110 catalytic activity [131]. A separate study showed that although mutants of p85α can bind all isoforms of p110, only p110α mediated oncogenic transformation [134]. It has been suggested that mutations in interacting domains of p85 and p110α may be functionally equivalent, for example mutations in the nSH2 domain of p85, such as the K379E mutation, may have the same effect as mutations such as the hotspot E542K and E545K mutations in the helical domain of p110α [8,134].

DUAL REGULATION BY p85 OF THE PTEN LIPID PHOSPHATASE, IN ADDITION TO THE p110 LIPID KINASE

The critical balance of PI3K/PTEN signalling to control the magnitude and duration of Akt pathway activation is disrupted in many cancers. This can occur through the gain-of-function mutational activation of p110α (noted above), or through the loss-of-function mutation or reduction of PTEN [135]. PTEN counteracts PI3K signalling by dephosphorylating PtdIns(3,4,5)P_3 lipids at the 3-position to prevent further activation of downstream Akt signalling [136]. The tumour suppressor function of PTEN has been expanded to include nuclear-localized PTEN specifically lost in several cancers [137].

Careful study of PTEN tumour suppression has established PTEN haploinsufficiency where loss of one *Pten* allele is sufficient to promote tumorigenesis [138,139]. Loss or mutation of one allele occurs at high frequency in primary human breast and prostate tumours, whereas mutation of the second allele occurs at lower frequency and is associated with advanced metastatic cancer [136]. $Pten^{+/-}$ mice develop a spectrum of neoplastic cancers of the breast, prostate and other tissues [140–142]. PIN (prostatic intraepithelial neoplasia) does not progress to invasive carcinoma in $Pten^{+/-}$ mice, indicating that PTEN heterozygosity is sufficient for tumour initiation, but not progression. Prostate-specific homozygous $Pten^{-/-}$ mice develop invasive carcinoma [143,144], suggesting that PTEN ablation is required for cancer progression. However, when combined with other genetic mutations (e.g. p53, $p27^{KIP1}$), $Pten^{+/-}$ mice develop invasive prostate cancer [145,146].

The role of PTEN dose in tumour formation and progression has been addressed with hypomorphic mice containing one *Pten* allele with an intronic neomycin cassette, $Pten^{hy}$. This allows transcriptional inference to reduce PTEN expression below wild-type ($Pten^{hy/+}$) and heterozygous ($Pten^{hy/-}$) levels [147,148]. $Pten^{hy/+}$ mice express 80 % of wild-type PTEN, show decreased survival, and develop mammary and endometrial tumours after a long latency. Several sets of genes involved in tumorigenesis were up-regulated in these mice, and were similar to those observed with human patients [147]. $Pten^{hy/-}$ mice express 30 % of wild-type PTEN, and develop high-grade PIN and locally invasive carcinoma at low penetrance, whereas $Pten^{+/-}$ mice only develop

low-grade PIN [148]. Thus reduction of PTEN below wild-type or heterozygous levels increases cancer development and progression respectively. It is tempting to speculate that alterations in processes regulating PTEN may have an impact on cancer susceptibility and progression.

Studies to determine the role of the individual subunits of p110α, p110β, p85α and PTEN unwittingly highlighted an important paradox in PI3K signalling: selective reduction of p85α unexpectedly improved insulin signalling. Some studies have knocked out all three products of the *Pik3r1* gene (p85α, p55α and p50α; *Pik3r1*$^{-/-}$), whereas others have selectively targeted only the large p85α isoform (p85$\alpha^{-/-}$). *Pik3r1*$^{-/-}$ results in perinatal lethality [149], whereas the selective p85$\alpha^{-/-}$ mice are viable [150]. These p85$\alpha^{-/-}$ mice have increased expression of both the p55α and p50α isoforms, and the p50α protein can bind p110 and tyrosine-phosphorylated proteins to partially compensate for the loss of p85 [150]. p110$\alpha^{-/-}$ and p110$\beta^{-/-}$ mice die at early embryonic stages through defects in the ability of cells to proliferate, whereas p110$\alpha^{+/-}$/p110$\beta^{+/-}$ mice are viable, suggesting that both isoforms of p110 perform vital distinct roles in development [151,152]. Homozygosity for a kinase-dead p110α was also embryonic lethal; however, heterozygous p110α-kinase-dead mice showed severe defects in insulin signalling and were glucose intolerant [153]. Mice heterozygous for either p110α or p110β have normal insulin signalling, whereas mice heterozygous for both are slightly glucose resistant and surprisingly have a decrease in total p85 levels [54]. If the regulation of p110α-mediated insulin signalling was the only function for p85 in this pathway, mice with either reduced p85 or p110α levels should respond to insulin similarly, but they do not [54]. Both *Pik3r1*$^{+/-}$ mice and p85$\alpha^{-/-}$ mice have increased glucose uptake and insulin sensitivity [149,150], more reminiscent of *Pten*$^{+/-}$ mice [154]. Thus over the past decade p85α has emerged as a regulator of PTEN, in addition to its better known regulation of p110-PI3K.

Insulin activates the IR, a tyrosine kinase that phosphorylates IRS-1, and recruits p85 to promote PI3K signalling. Insulin-resistant diabetic *Isr*$^{+/-}$/*Irs1*$^{+/-}$ mice showed improved insulin signalling when also heterozygous for p85 [155]. Cells derived from p85$\alpha^{+/-}$ mice had similar amounts of p85 complexed to p110 and net PI3K activity as compared with p85$\alpha^{+/+}$ wild-type cells [156]. Lysates from p85$\alpha^{+/-}$ cells also contained some p85 after immunodepletion of p110, indicating that p85 may exist in excess of p110. These studies suggest that p85 preferentially binds p110 and, once saturated, free p85 binds other partners that negatively regulate insulin/PI3K signalling.

Two models have emerged to describe negative regulation of PI3K signalling by p85α: sequestration of IRS-1 [157] and regulation of PTEN [158]. In the sequestration model, free p85 competes with p85–p110-PI3K for phosphorylated IRS-1, sequestering IRS-1 in non-signalling complexes [58,159]. In support of this model, transgenic expression of human placental growth hormone in mice increased p85α expression and reduced IRS-1-associated p110-PI3K activity in response to insulin, causing severe insulin resistance [58].

In the second model, p85α negatively regulates PI3K signalling by binding to and enhancing PTEN function. PTEN is regulated by several post-translational modifications as well as by p85α (Figure 2). Serine/threonine phosphorylation within the C-terminal regulatory domain of PTEN is thought to promote a closed conformation [160] through ionic interactions between the charged phosphate groups and a surface of basic residues within the phosphatase (PASE) and C2 domains of PTEN. In this form, PTEN is proposed to be monomeric, inactive and located within the cytosol. Upstream activating signals cause dephosphorylation of PTEN within the regulatory domain, inducing an open conformation. The newly exposed basic residues within the PASE and C2 domains of PTEN, as well as its N-terminal PtdIns(4,5)P_2-binding motif, facilitate plasma-membrane association through electrostatic interactions [160–164]. The p85 protein binds PTEN that is unphosphorylated at the regulatory serine/threonine phosphorylation sites (Figure 2) [165]. The p85–PTEN interaction is direct and requires EGF stimulation in HeLa cells [93], likely to induce PTEN dephosphorylation. Ethanol treatment can also induce a rapid association of p85 and PTEN, but not p110, in a model of hepatic injury [166]. The lipid phosphatase activity of PTEN is increased approximately 3-fold by p85 binding in an *in vitro* assay [93], suggesting that p85 is a positive regulator of PTEN function. Liver cells derived from liver-specific *Pik3r1*$^{-/-}$ mice showed decreased PTEN activity, increased Akt activation and elevated PtdIns(3,4,5)P_3 levels, suggesting a defect in lipid clearance [167]. These results support a role for p85α in PTEN regulation in cells.

The C-terminal PDZ-binding motif of PTEN can bind several large membrane-associated scaffolding proteins through interaction with their PDZ domains [168]. This high molecular mass ($\sim$670 kDa) PAC (PTEN-associated complex) [160] also contains p85α and p110β proteins [165] and is thought to organize signalling complexes to promote their efficiency. The p85 protein co-immunoprecipitates with PTEN in PAC fractions isolated by gel-filtration chromatography. Interestingly, p110β co-immunoprecipitates with the p85–PTEN complex, although it is not clear whether p110β associates directly with p85–PTEN or indirectly via PAC-associated scaffold proteins [165]. The isolated GAP domain of p85 was capable of binding PTEN, although deletion of both the N-terminal SH3 and GAP domains of p85 were required to prevent the PTEN interaction [93]. This N-terminal half of p85 is absent from the p55α/p50α isoforms, and expression of a p85 mutant missing these domains (i.e. p85$\triangle$SH3-GAP, similar to the p50α isoform) increased both the magnitude and duration of PDGF-stimulated Akt activation [93].

Taken together, p85 directly binds and positively regulates the lipid phosphatase activity of active unphosphorylated PTEN (Figure 2). Depletion of p85, as is found in p85$\alpha^{+/-}$ cells, is speculated to reduce the pool of p85 interacting with and positively regulating PTEN, without significantly having an impact on the level of p85–p110-PI3K complex [156]. The reduced PTEN activity increases Akt activation and improves insulin sensitivity (Figure 2) [94]. Maintaining sufficient p85 to form p85–PTEN complexes may have important implications for cancer development and progression. The incidence of intestinal polyps increased 2-fold in *Pten*$^{+/-}$/p85$\alpha^{+/-}$ mice compared with *Pten*$^{+/-}$ mice; however, these neoplasms did not progress to invasive carcinoma [169]. Mice with a liver-specific *Pik3r1*$^{-/-}$ not only showed decreased PTEN activity, elevated PtdIns(3,4,5)P_3 levels and increased Akt activation, they also developed liver tumours which progressed to metastatic cancer [170]. Ablation of p85α was sufficient to drive cancer progression in this model. Thus reduction of p85 levels, a positive regulator of PTEN activity and function, sufficiently below normal could promote cancer susceptibility and progression, particularly in cells with reduced PTEN.

ROLE FOR PI3Ks IN RECEPTOR TRAFFICKING

The importance of class IA PI3Ks in signalling downstream from cell-surface receptors is highlighted by the study of mutant PDGFRs (Y740F + Y751F) that lack the two tyrosine residues required for p85 binding. These mutant PDGFRs are unable

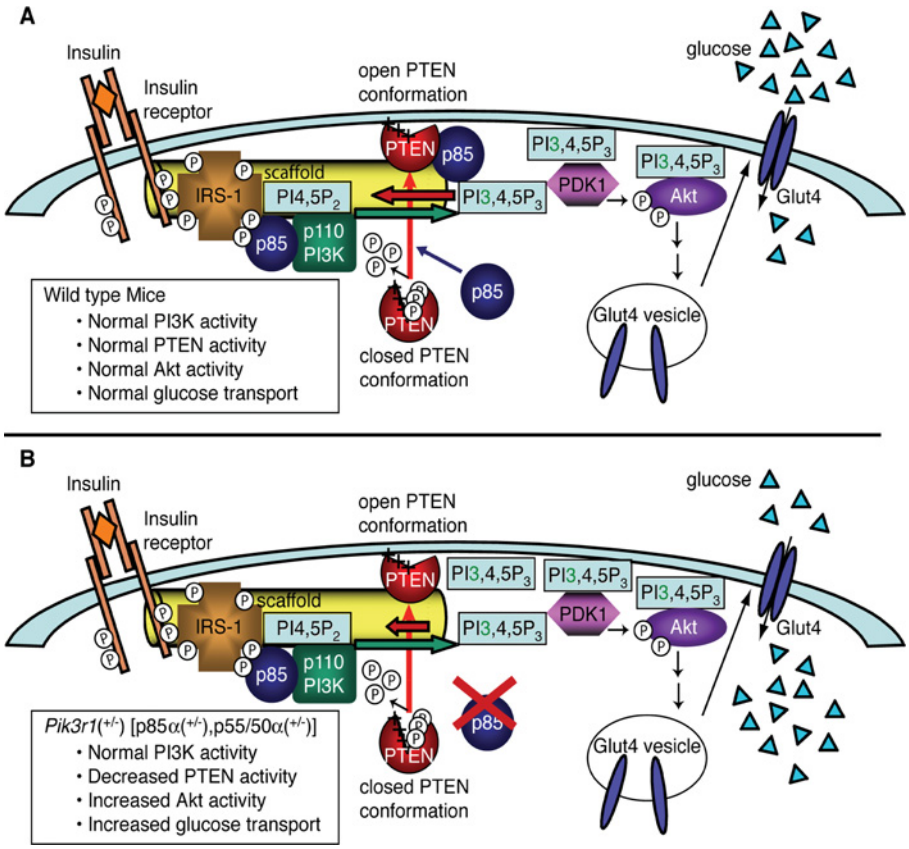

Figure 2 Model for modulation of insulin signalling via the p85α–PTEN interaction

(**A**) Insulin-mediated activation of the IR induces phosphorylation of IRS-1 and recruitment of p85α–p110-PI3K. Phosphorylation of PtdIns(4,5)P_2 by activated membrane-localized PI3K generates PtdIns(3,4,5)P_3, which leads to the recruitment and activation of PDK1 and Akt kinases. Signalling downstream of Akt causes trafficking of Glut4 (glucose transporter 4)-positive vesicles to the plasma membrane and import of extracellular glucose. Dephosphorylation of the PTEN regulatory domain transitions cytoplasmic PTEN from an inactive closed conformation to an active open conformation. This exposes several basic residues within PTEN, facilitating electrostatic interactions with the plasma membrane. PTEN is found in a large PAC containing several other proteins, including scaffolding proteins and p85. Interaction with p85 increases PTEN lipid phosphatase activity, improving efficiency of PtdIns(3,4,5)P_3 dephosphorylation to ensure transient PI3K signalling. (**B**) Mice heterozygous for *Pik3r1* [p85$\alpha^{+/-}$ and p55/50$\alpha^{+/-}$] and wild-type mice show similar levels of p85α–p110-PI3K and IRS-1-associated PI3K activity in response to insulin. Heterozygosity of p85α selectively depletes the pool of p85 not associated with p110, disrupting the p85α–PTEN interaction. PtdIns(3,4,5)P_3 levels are increased due to suboptimal PTEN phosphatase activity, leading to increased Akt activation and signalling, resulting in enhanced glucose transport. Heterozygosity of p85α improves insulin sensitivity and glucose transport via selective reduction of p85-stimulated PTEN phosphatase activity.

to activate the PI3K pathway in response to PDGF, blocking many cellular processes, including migration and survival [171]. In addition, PDGFRs that lack binding sites for p85 failed to be down-regulated correctly [171]. Wild-type PDGFRs are internalized into endosomes and trafficked through the endocytic pathway [172]. Some fraction of internalized receptors are sorted to a lysosomal degradative pathway, and the remaining receptors are deactivated and recycled back to the plasma membrane. Non-p85-binding (Y740F + Y751F) PDGFRs were internalized with similar efficacy as wild-type receptors; however, the mutant receptors were not trafficked to the lysosome for degradation, but were instead all recycled to the cell surface [173]. The lack of mutant (Y740F + Y751F) PDGFR degradation caused sustained activation of some downstream signalling pathways. A subsequent study showed that in their unphosphorylated state, these YxxM motifs also served as tyrosine-based endocytic-sorting motifs, independent of p85–p110 binding [174], suggesting dual functions in PI3K signalling and sorting for these motifs.

There are two main pathways used during receptor-mediated endocytosis to internalize activated receptor complexes and initiate trafficking to the early/sorting endosome, clathrin-mediated endocytosis and caveolin-mediated endocytosis (also known as non-clathrin-mediated endocytosis) (reviewed in [22,175]). Endocytic vesicles are internalized from the plasma membrane through scission events mediated by the large GTPase dynamin, whose activity is up-regulated by p85 SH3 domain binding [69]. The trafficking of activated RTK complexes within the resulting vesicles is regulated by small monomeric Rab GTPases that control vesicle fusion and budding events [92,176,177]. There are more than 60 Rabs in mammalian cells responsible for regulating secretion and endocytosis, with each Rab regulating specific membrane-trafficking events between various compartments, including endoplasmic reticulum, Golgi stacks, plasma membrane, early endosomes, late endosomes and lysosomes (reviewed in [90–92,178,179]).

Rab GTPases are regulated by their cytosolic compared with membrane localization and nucleotide-bound state. C-terminal prenylation of Rab proteins facilitates membrane association. In contrast with many other monomeric G-proteins that are constitutively membrane-associated (e.g. Ras), Rab proteins can shuttle between membrane-bound and cytosolic locations through masking of their prenyl group by binding to a GDI (guanine-nucleotide-dissociation inhibitor) protein. Cytosolic Rab proteins are bound to GDP in an inactive conformation that is stabilized by GDI protein binding. GDFs (GDI displacement factors) are membrane-bound protein factors important for the membrane

recruitment of Rab-GDP through their ability to displace GDI proteins and expose the prenyl groups for membrane association.

GEFs (guanine-nucleotide-exchange factors) activate Rab proteins by stimulating the release of GDP and binding of the more abundant GTP. GTP binding induces a conformational change within the flexible switch I and II regions to produce the active Rab-GTP conformation capable of interacting with a variety of downstream effector proteins. GAPs stimulate the weak intrinsic GTPase activity of the Rab protein typically 100–1000-fold. GAP proteins typically function to both stabilize the switch regions of the GTP-bound form and also provide a catalytic arginine, termed an 'arginine finger', which stabilizes the transition state during GTP to GDP hydrolysis [180].

Rab5 is one of the most widely studied Rab-family members and plays a critical role in endosome fusion during receptor-mediated endocytosis to traffic activated RTK complexes from the plasma membrane to the early/sorting endosome (reviewed in [91,92,176]). Most RTKs are deactivated and recycled from the early/sorting endosomes back to the plasma membrane, by a rapid Rab4-dependent process or via a slower Rab4- and Rab11-dependent process [91,92,176]. A fraction of RTKs are sorted from the early/sorting endosome into multivesicular bodies within the late endosome. Receptor degradation requires monoubiquitination of the receptor by the Cbl E3 ubiquitin ligase [91,92,176]. A complex series of sequential interactions then sorts monoubiquitinated receptor/cargo proteins into the intralumenal multivesicular bodies through the action of ESCRT (endosomal sorting complex required for transport) proteins [181,182]. These late-endosomal compartments fuse with lysosomes, through a Rab7-dependent process, giving rise to receptor degradation. Disruptions in Rab-mediated endocytosis can perturb receptor trafficking and downstream signalling, resulting in oncogenesis [183–186].

Rab5 mediates the homotypic fusion of early endosomes containing activated receptor complexes to form larger early/sorting endosomes (Figure 3). To accomplish this function Rab5-GTP binds numerous effector proteins, some of which have key roles in vesicle tethering and vesicle fusion events. EEA1 (early endosomal antigen 1) is a critical tethering factor that forms a bridge between two vesicles by binding to Rab5-GTP and the lipid PtdIns3*P* [187–189]. Rab5-GTP also binds components of the vesicle fusion machinery, including the SNARE [soluble NSF (*N*-ethylmaleimide-sensitive fusion protein)-attachment protein receptor] complex proteins syntaxin13, syntaxin6 and the ATPase NSF [190,191].

The majority of PtdIns3*P* is synthesized by class III p150–Vps34-PI3Ks, another Rab5-GTP-binding effector [192]. However, class II PI3Ks have also been shown to synthesize PtdIns3*P* in response to stimuli and are now thought to play a significant role in dynamin-dependent and -independent endocytosis [193–196]. Rab5 binds p85α [84] and in its GTP-bound form binds p110β [192], providing a mechanism to localize Rab5 to sites of PtdIns(3,4,5)P_3 generation. Two lipid phosphatases, PI4-Pase (PI-4-phosphatase) and PI5-Pase (PI-5-phosphatase) [197] are Rab5-GTP effector proteins that may sequentially dephosphorylate PtdIns(3,4,5)P_3 to generate PtdIns3*P*, as an additional mechanism to contribute the necessary PtdIns3*P* for endocytic processes. In support of this contribution, antibodies targeting p110β result in a 30% reduction in PtdIns3*P* production, comparable with the reduction seen when a PI4-Pase-blocking antibody was used, and leading to the hypothesis that p110β and PI4-Pase contribute to a common pathway of PtdIns3*P* generation [197]. This suggests that in addition to acting as a second messenger for downstream signalling, PtdIns(3,4,5)P_3 produced by p110 may serve as a substrate for PI4-Pase and PI5-Pase producing PtdIns3*P*, which can bind to EEA1, facilitating membrane tethering.

The p85 protein can also influence Rab5-mediated cell migration through effects on β-integrin trafficking. Rab5, as well as Rab21, can regulate endocytosis and recycling of β-integrins and have key roles in cell adhesion and migration [198]. The internalization of β-integrins from focal adhesion sites mediating the attachment of cells to the extracellular matrix at the lagging edge of cells, allows integrins to be relocated to the leading edge of cells during migration as they form new attachments. During cell migration, Rab5 has also been shown to organize actin structures [199], acting together with PI3K and Rac1, but also independently of them [200,201]. Caspase 8 can also promote cell migration, in addition to its roles in proliferation and apoptosis. Tyrosine-phosphorylated caspase 8 binds p85, sequestering its GAP activity away from Rab5, resulting in increased Rab5-GTP [48] and enhanced trafficking of integrins.

In the context of RTK-mediated endocytosis, there are some Rab5 regulatory proteins that specifically bind to activated receptors to provide highly localized receptor/cargo-specific functions (Figure 3). The p85 protein binds to activated RTKs and can also bind Rab5-GDP [84], and thus may serve as a GDF to identify the appropriate membranes for Rab5-GDP association. Rin1 proteins bind to activated RTKs [202–204] and are Rab5 GEFs that promote Rab5 nucleotide exchange to generate Rab5-GTP. This initial Rab5-GTP may subsequently bind another Rab5-GTP effector, the Rabaptin5–Rabex5 complex in which Rabaptin5 binding to Rab5-GTP localizes and activates the Rab5 GEF Rabex5 to further activate additional Rab5-GDP proteins through nucleotide exchange to Rab5-GTP [205,206].

As noted above, the p85 protein is also a Rab5 and Rab4 GAP, stimulating the Rab-mediated hydrolysis of GTP to GDP [84]. Mutation of the arginine finger within the GAP domain of p85 (R274A) prevents Rab GAP activity [84], and enhances Rab5-mediated endocytosis and Rab4-mediated recycling pathways. Expression of the p85-R274A mutant in cells caused enhanced receptor activation and downstream MAPK (mitogen-activated protein kinase) signalling, with reduced receptor degradation [84] resulting in cellular transformation [207]. Receptors in these cells were rapidly trafficking through the early/sorting endosomal compartment, with reduced opportunities for sorting into late endosomal/lysosomal compartments and reduced opportunities for deactivation/dephosphorylation [89].

GAP proteins typically provide two functions to enhance G-protein-mediated GTP hydrolysis, an arginine finger and switch stabilization functions. The ability of p85 to bind to both Rab5-GDP and Rab5-GTP [84], suggests that p85 is not likely to interact with and stabilize the Rab5 switch regions since they adopt very different conformations in the two nucleotide-bound states. The ability of p110β to bind selectively to Rab5-GTP [192] instead suggests that it could provide the second GAP regulatory function of switch stabilization. This is consistent with the suggestion that p110β may contribute protein scaffolding functions independent of lipid kinase activity [114,208]. Cells devoid of p110β have defects in EGFR trafficking (another receptor that can be degraded in the lysosome, as well as be deactivated and recycled) and transferrin uptake (a receptor that is always recycled), with defects in clathrin-coated pit formation [208]. Re-expression of a kinase-dead p110β rescues this trafficking defect, whereas expression of an activated Rab5 (Q79L) does not [208,209]. Collectively, these results suggest that p85α and p110β contribute to the co-ordination of RTK trafficking on multiple levels, through the local generation of PtdIns(3,4,5)P_3 lipids (subsequently converted into PtdIns3*P*), through the Rab GAP activity of p85, and possibly via emerging Rab switch stabilization (i.e. scaffolding) functions for

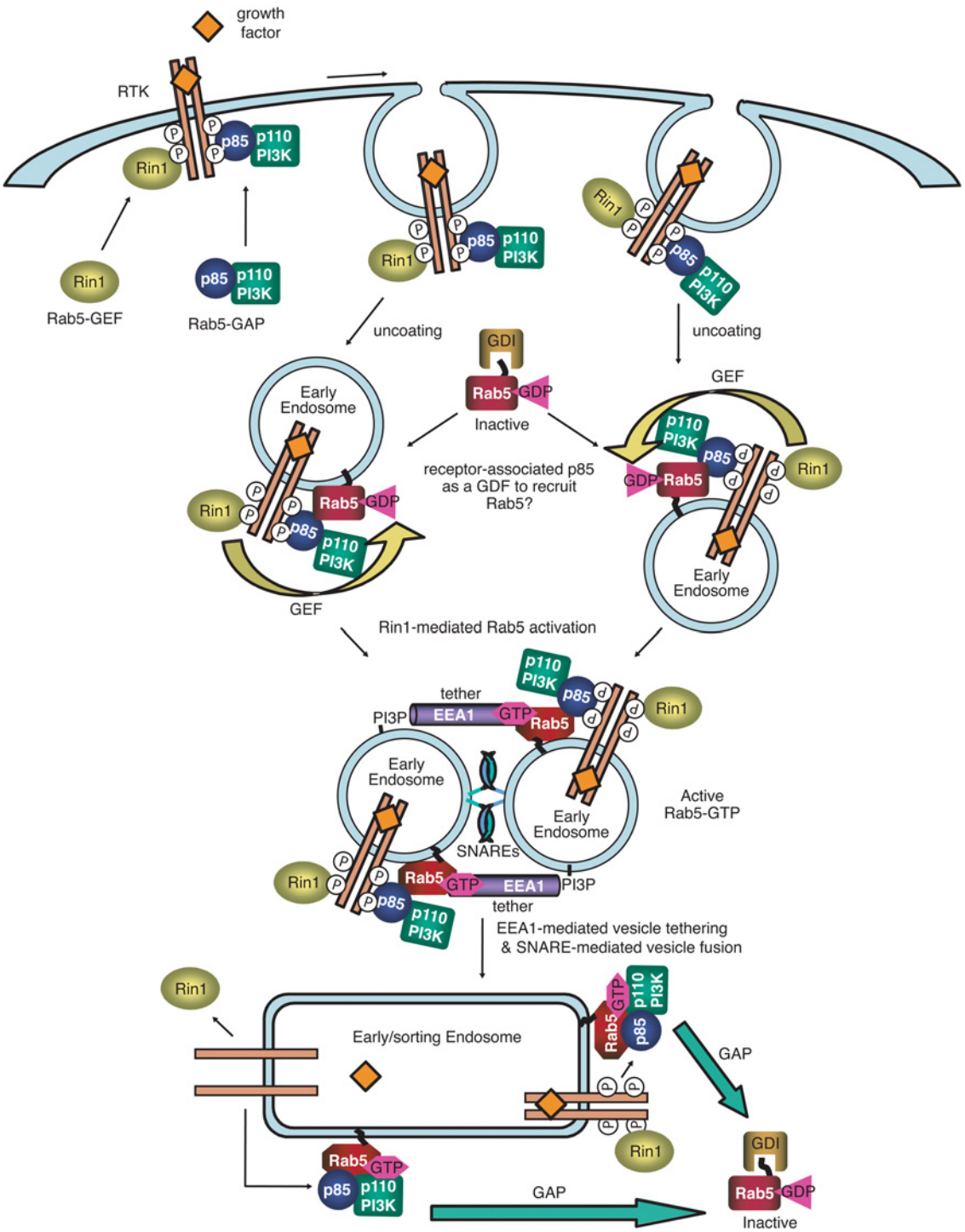

Figure 3 Model for receptor-associated proteins in the regulation of Rab5-mediated processes

RTKs activated by growth factor binding become tyrosine phosphorylated and recruit Rin1 (a Rab5 GEF) and p85α–p110β (a possible Rab5 GDF and a Rab5 GAP). RTK complexes are internalized into early endosomal vesicles by clathrin-dependent and clathrin-independent processes, which involve dynamin-dependent scission from the plasma membrane and the subsequent uncoating steps (not shown). The ability of p85 to bind Rab5-GDP suggests that RTK-associated p85 could act as a GDF to identify the appropriate vesicle membranes for Rab5 recruitment. The RTK-associated Rin1 is a Rab5 GEF that generates active Rab5-GTP suitable for interactions with many effector proteins (discussed in the text). One effector protein is EEA1, which forms a tether between two early endosomes by binding to Rab5-GTP on one endosome and PtdIns3*P* lipids on a second endosome, to facilitate SNARE-mediated vesicle fusion. Rab5-GTP is no longer required within the larger early/sorting endosome. We propose that p85α–p110β dissociates from the RTK, up-regulating its GAP activity towards Rab5-GTP to promote the formation of inactive Rab5-GDP, and allowing for GDI binding and membrane release. An animated version of this Figure is available at http://www.BiochemJ.org/bj/441/0023/bj4410023add.htm.

p110β and/or the identification of RTK-containing membranes for Rab5 recruitment through Rab5-GDP binding to p85α.

An emerging model within the vesicle trafficking and endocytosis field is that proteins associate/dissociate with vesicle membranes and some of these may include lipid kinases and phosphatases to alter the membrane lipid composition [92,175]. These changes in protein and lipid composition of the vesicle cause it to 'convert' from one vesicle type to another or 'mature' by altering the identity and function of the vesicle. As activated RTKs are trafficking through the endocytic pathway, they bind not only signalling proteins, but proteins capable of regulating Rab5-mediated trafficking functions (Figure 3). For example, both EGFR and PDGFR associate with p85, a possible GDF for Rab5 since p85 can bind Rab5-GDP [84]. Receptor-associated p85 would serve as a cargo-specific GDF, an ideal mechanism to identify membranes containing activated RTKs for Rab5 association. A cargo-specific GDF may also explain why so few GDFs have been identified to date, since these studies have primarily been carried out in yeast that lack RTKs and their associated regulatory proteins.

Activated RTKs also bind Rin1 [202,204,210], a GEF for Rab5, which can convert inactive Rab5-GDP into active Rab5-GTP (Figure 3). This priming event could provide some Rab5-GTP to further recruit and activate additional Rab5-GTP through the binding of Rabaptin5–Rabex5 (a Rab5-GTP adapter and Rab5 GEF), proposed to form a positive-feedback loop. Other key Rab5-GTP effectors such as p150/Vps34 (to generate PtdIns3*P*) and p85α–p110β plus PI4-Pase and PI5-Pase [to generate PtdIns(3,4,5)P_3 and convert it into PtdIns3*P*] could provide high local concentrations of PtdIns3*P* lipids. The binding of EEA1 to both Rab5-GTP and PtdIns3*P* tethers early endosomes together for SNARE-mediated fusion (Figure 3). Rab5-mediated recruitment of the HOPS (homotypic fusion and vacuole protein sorting) complex containing Vps39, a GEF for Rab7, may then recruit and activate Rab7, a late endosomal Rab [211]. It has been proposed that a Rab5 GAP may also be recruited to generate Rab5-GDP and promote GDI-mediated membrane extraction of Rab5 to convert the vesicle from a Rab5-positive early endosome into a Rab7-positive late endosome [211].

It is also possible that after vesicle fusion RTKs may release p85α–p110β, perhaps via RTK dephosphorylation. It is possible that p85–p110, in its receptor-free state, would allow both p85 and p110β to bind Rab5-GTP and exert full GAP activity (i.e. p85, arginine finger; p110β, switch stabilization). In support of this model, we have observed that two p85 mutants remain bound to activated PDGFRs up to 4 h after PDGF stimulation, long after wild-type p85 has dissociated. One such p85 mutant is the arginine finger mutant p85-R274A that selectively binds to Rab5-GDP, and not Rab5-GTP [84]. The second is p85$\triangle$110, a p85 mutant unable to bind p110 [212], but that can bind to both Rab5-GDP and Rab5-GTP (D.H. Anderson, unpublished work). These results suggest that the ability of p85 to bind Rab5-GTP and p110 are both requirements for the release of p85 from the PDGFR. This model provides an organized, integrated and sequential mechanism for the regulation of Rab5-mediated receptor trafficking, with important roles for p85.

CONCLUDING REMARKS

The p85 protein interacts with a large complex network of proteins that provide opportunities to co-ordinate several cellular processes in response to upstream receptor activation. These processes include actin reorganization and receptor trafficking, as well as PI3K signalling pathway activation and deactivation. Mutations within the C-terminal half of p85α have been identified in human tumour samples, and many of these conferred a gain-of-function phenotype through maintained binding and stabilization of p110, yet had lost p110-PI3K inhibitory function. With the recent discovery of key proteins that bind to the N-terminal half of p85 (e.g. PTEN and Rab5) that can influence the dephosphorylation of PI3K lipid products (PTEN) or receptor trafficking and signalling functions (Rab5), we anticipate that cancer-associated mutations within this region of p85 will also be uncovered.

The newly identified p85–PTEN interaction may be a double-edged sword; reduction of interaction may improve glucose homoeostasis, but increase cancer incidence and severity. Pharmacological disruption of p85–PTEN could improve insulin sensitivity by increasing Akt activation and glucose transport, since heterozygosity of p85α reduced the incidence of diabetes in insulin-resistant mice [155]. Yet in combination with reduced PTEN, p85$\alpha^{+/-}$ results in enhanced tumour formation [169]. Thus the challenge for the future will be to strike a delicate balance between positive p85–p110-PI3K and negative p85–PTEN-PI3Pase effects to promote appropriate and transient PI3K signalling responses.

Note added in proof (received 13 October 2011)

After submitting this paper, *Pik3r1* mutations (encoding p85α) were reported at high frequency (20 %; 48/243) in endometrial cancer [213]. Interestingly, *Pik3r1* mutations were frequently present in endometrial cancers with reduced PTEN levels, and this combination resulted in increased pAkt levels, comparable with that in PTEN-null cells. The authors further demonstrated that wild-type p85α monomers interact with p110α, and that excess p85α forms homodimers that from separate complexes with PTEN. The p85α–PTEN complex serves to stabilize PTEN by blocking PTEN ubiquitination and its subsequent proteasomal degradation. Formation of the p85α–PTEN complex was blocked by a patient-derived truncation mutant of p85α (E160*) that interferes with p85 homodimerization, resulting in PTEN destabilization. These findings further support an emerging critical role for p85α in the regulation of PTEN.

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We apologize to those whose work could not be cited due to space constraints.

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