Minireview

Phosphoinositide 3-kinase-dependent activation of Rac

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Abstract The monomeric GTPase Rac and the lipid kinase phosphoinositide 3-kinase (PI3K) are intracellular signalling enzymes that each regulate a huge range of cellular functions. Their signalling pathways overlap. Several pathways lead from PI3K activation via the production of the lipid second messenger phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)P_3) to the activation of guanine-nucleotide exchange factors (GEFs) that activate Rac. Vice versa, Rac can also stimulate the activation of PI3K, although the mechanism for this is unclear. We review here the evidence that links PI3K and Rac signalling pathways.
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Key words: Rac; GTPase; Phosphoinositide 3-kinase; Guanine-nucleotide exchange factor; Signalling

1. Introduction

Rac proteins (Rac1, 2 and 3) are a subfamily of the Rho family of monomeric GTPases. They regulate a wide range of universally important cellular functions including the structure of the actin cytoskeleton, cell/cell contacts and adhesion, transcriptional and translational activation, protein synthesis, cell survival and cell cycle entry [1,2]. In addition, Rac proteins also control cell type-specific functions such as regulated secretion in mast cells, reactive oxygen species formation by the NADPH oxidase complex in neutrophils, and axon guidance in neurones [1].

All Rho family GTPases, including Rac proteins, are molecular switches that are inactive when guanosine diphosphate (GDP)-bound and active when guanosine triphosphate (GTP)-bound [3]. When GTP-bound, they bind to their target proteins; for Rac, about 15 direct targets are currently known [1]. In resting cells, Rac-GDP is inactive, cytosolic, and generally bound to a GDP dissociation inhibitor (GDI). After dissociation of the GDI (the regulation of this step is still unclear), Rac-GDP attaches to the membrane via its C-terminal prenylation. This allows binding of the activating enzyme, a guanine-nucleotide exchange factor (GEF). The GEF prises open the GTPase's nucleotide-binding site, allowing dissociation of GDP and binding of GTP (which is present at much higher concentration than GDP in the cytosol of the cell). Inactivation occurs by hydrolysis of bound GTP to GDP through the intrinsic GTPase activity of Rac and can be accelerated by a GTPase-activating protein. Although Rac activation can theoretically occur at several steps, i.e. removal of the GDI, recruitment or activation of the GEF and removal or inhibition of the guanosine triphosphatase-activating protein (GAP), the available evidence suggests that it depends largely on the activation of the GEF [3] (Fig. 1).

Phosphoinositide 3-kinase (PI3K) family enzymes phosphorylate the phosphoinositide class of lipids at the 3-OH position of the inositol ring [4]. Among them, type 1 PI3Ks are regulated by stimulation of cell surface receptors. Type 1A PI3Ks (PI3Kα, β and δ) are activated by receptor-tyrosine kinases and intracellular protein-tyrosine kinases, whereas type 1B PI3K (PI3Kγ) is activated by G protein-coupled receptors via the βγ subunits of heterotrimeric G proteins. Both type 1A and type 1B PI3Ks are also activated by Ras. Upon activation, all type 1 PI3Ks phosphorylate phosphatidylinositol (4,5)-diphosphate (PtdIns(4,5)P_2), a constitutive membrane component, to create the lipid second messenger phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)P_3) (and its immediate metabolic product PtdIns(3,4)P_2). The PtdIns(3,4,5)P_3 signal is mediated by its binding to the plextrin homology (PH) domain of target proteins, which induces their membrane translocation and/or conformational changes. Known PtdIns(3,4,5)P_3 targets are numerous and from a variety of enzyme classes, e.g. protein kinases, phospholipases and GEFs [4]. These PtdIns(3,4,5)P_3 targets together regulate a huge list of essential cellular functions including transcription, translation, protein synthesis, cell survival, cell cycle entry and the structure of the actin cytoskeleton [4].

2. PI3K upstream of Rac

The wide overlap in cellular functions regulated by Rac and type 1 PI3Ks has long suggested a link between their signalling pathways. Indeed, there is now a large body of evidence showing that PI3K can activate Rac indirectly via PtdIns(3,4,5)P_3-sensitive Rac-GEFs, as will be described below. PtdIns(3,4,5)P_3 can also bind directly to Rac in vitro, and at high concentration (EC_{50} around 50 μM) PtdIns(3,4,5)P_3 can facilitate the dissociation of GDP from

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Abbreviations: PI3K, phosphoinositide 3-kinase; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5)-triphosphate; GEF, guanine-nucleotide exchange factor; GDI, guanosine diphosphate dissociation inhibitor; DH, Dbl homology; PH, plextrin homology

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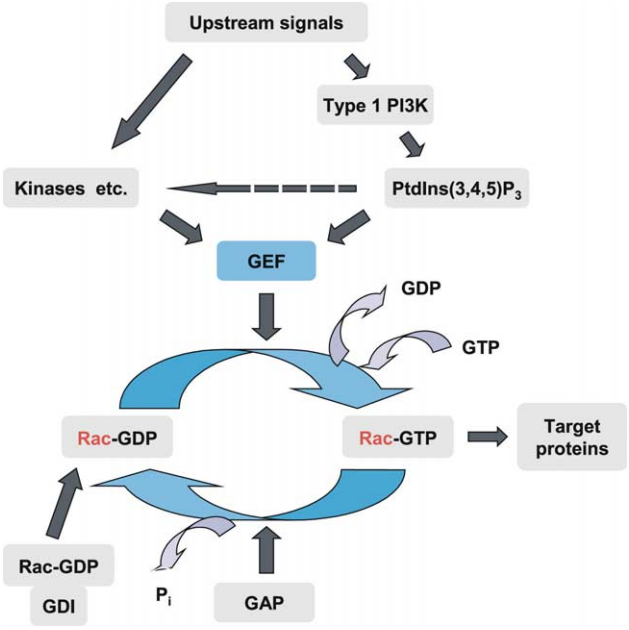

Fig. 1. Mechanisms of Rac activation. Signals for the activation of Rac (=exchange of GDP for GTP) can be transmitted in PI3K-dependent and in PI3K-independent ways via the activation of GEFs. Several families of Rac-GEFs can be activated directly by the lipid product of PI3K activity, PtdIns(3,4,5)P_3.

Rac [5]. However, PtdIns(3,4,5)P_3 preferably binds and stabilises the nucleotide-free form of Rac and does not promote GTP loading [5]. Thus, the significance of this interaction for the regulation of Rac in vivo is still unclear.

First indications that type 1A PI3K can be upstream of Rac activation came from experiments using the PI3K inhibitor wortmannin and dominant-negative PI3K constructs. They were shown to inhibit the Rac-dependent pathways of platelet-derived growth factor (PDGF) and insulin-stimulated membrane ruffling, and overexpression of wild type or constitutively active Rac could override this inhibition [6–8]. Formal proof that type 1A PI3K is a necessary signal for Rac activation in some situations came from direct measurements of PDGF-stimulated, wortmannin-sensitive Rac-GTP loading in a Rac-overexpressing endothelial cell line [7]. Later, co-expression of various combinations of Gβγs, type 1B PI3K, and the Rac-GEF Vav in COS cells has revealed that type 1B PI3K is also capable of activating Rac, and implicated an unidentified Rac-GEF as the mediator [9].

Since these studies, extensive use of PI3K inhibitors and dominant-negative PI3K mutants has shown that PI3K is necessary for Rac activation in a large number of different systems. Equally, the use of dominant-negative Rac mutants has revealed that Rac mediates many of the PI3K-dependent pathways. However, it is very obvious that Rac does not mediate all PI3K pathways, a classic example of a PI3K-dependent but Rac-independent response being insulin-stimulated glucose transport [10]. Inversely, it is also clear that Rac cannot only be activated by PI3K. Some Rac-dependent cellular responses, such as membrane ruffling in bombesin-stimulated Swiss 3T3 cells [11], or activation of transcription factor AP1 in T cell receptor (TCR)-stimulated Jurkat T cells [12], are insensitive to PI3K inhibitors. In fact, multiple pathways of Rac activation seem to co-exist within any cell type. For example, in neutrophils, PMA can stimulate Rac2 activation in a wortmannin-insensitive manner whereas *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated Rac2 activation is wortmannin-sensitive, suggesting the co-existence of PKC- and PI3K-dependent activators of Rac2 in these cells [13]. The PI3K dependence of fMLP-stimulated Rac activation in neutrophils has been controversial. In contrast to the study be Akasaki et al. [13], two other equally convincing studies found it to be PI3K-independent [14,15], a main obvious difference being the times of stimulation studied. This apparent contradiction might now be explained by the discovery of P-Rex1, which we now know can receive both PI3K-dependent and PI3K-independent regulatory inputs ([16], and see below). However, the relative importance of PI3K-dependent versus PI3K-independent pathways of Rac activation for a given downstream response is still unclear in most circumstances.

3. PI3K-dependent Rac-GEFs

More than a dozen Rac-GEFs have been identified so far, some of them specific for the Rac family, some more promiscuous [3]. Among them, members of the Vav, Sos, Tiam, PIX, SWAP-70 and P-Rex families have been suggested to be regulated by PI3K in vivo. In vitro, PtdIns(3,4,5)P_3 directly and strongly activates the Rac-GEF activities of P-Rex1 and SWAP-70 [16,17], weakly (about two-fold) those of Vav1, Sos1 and possibly Tiam1 (although the latter is controversial) [18–21], but probably not that of αPIX [22] (Fig. 2). The abilities of the other known Rac-GEF families (namely Trio, Ras-GRF, Asef, Gef-H1 and Fir) to be activated by PI3K in vivo and/or to bind PtdIns(3,4,5)P_3 in vitro have not yet been studied.

Like all GEFs for Rho family GTPases, Rac-GEFs have tandem Dbl homology (DH)/PH domains, the DH domain being the catalytic guanine-nucleotide exchange domain. The PH domains in these DH/PH tandems do not conform to the consensus sequence for specific binding of PI3K products: they all substitute some of the conserved basic residues with others, including acidic amino acids [23]. Functionally, the PH domain in the DH/PH tandem differs from those that specifically bind PI3K products, such as in PKB and Dapp1, in one important aspect: wherever investigated so far, phosphoinositide binding to the PH domain in the DH/PH tandem is not sufficient to target the Rac-GEF to the membrane. So, while membrane localisation of the GEF is generally accepted to be a prerequisite for Rac activation, this is achieved through domains other than the PH domain in the DH/PH tandem, although that might cooperate with other membrane targeting mechanisms. In the cases of Sos1 and Vav1, PtdIns(3,4,5)P_3 binding to the PH domain is believed to relieve an intramolecular inhibition that allows activation [18,19,24]. However, the precise roles of the different GEF PH domains clearly need to be studied further.

3.1. Vav

The Vav family has three members, Vav1, 2 and 3. The regulation of Vav1 is the most intensely studied of all Rac-GEFs. Upon stimulation of a variety of membrane receptors, Vav1 is phosphorylated by intracellular protein-tyrosine kinases of the Src or Syk families [25], and this tyrosine phosphorylation is sufficient to activate Vav1 Rac-GEF activity in vitro and in vivo [26]. The nuclear magnetic resonance (NMR)

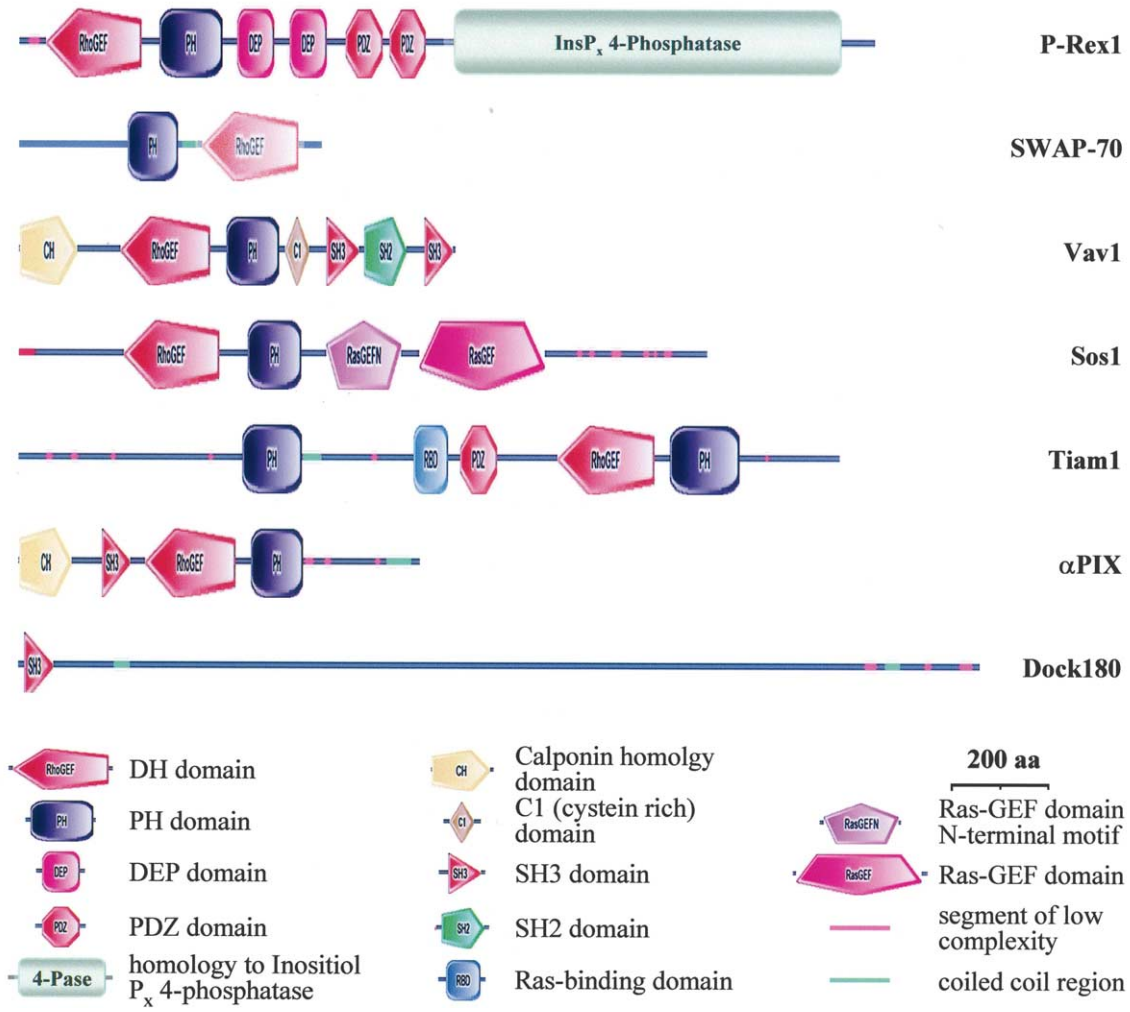

Fig. 2. Domain structures of Rac-GEFs that are activated by PI3K and/or bind PI3K products. The domain predictions were made using SMART (http://smart.embl-heidelberg.de). The inositol polyphosphate 4-phosphatase homology of P-Rex1 and the DH domain of SWAP-70 were added manually, as their homologies are too weak for SMART. The Rac-GEF activities of P-Rex1 and SWAP-70 are directly and strongly activated by PtdIns(3,4,5)P_3, whereas Vav1, Sos1 and (perhaps) Tiam1 are activated weakly. αPIX and Dock180 are probably not activated directly by PtdIns(3,4,5)P_3.

structure of a fragment of Vav1 revealed that this phosphorylation relieves an autoinhibitory interaction between Y174 and the DH domain [27]. PI3K is thought to modulate the activation of Vav1 by influencing its degree of tyrosine phosphorylation. A widely accepted model is that PtdIns(3,4,5)P_3 binding to Vav1 relieves an intramolecular interaction between PH and DH domains, thus facilitating tyrosine phosphorylation on Y174 and so further opening of the DH/PH domains, binding of Rac-GDP and catalysis [18,19]. How important though is the input of PI3K to Vav1 regulation in vivo? Experiments with PI3K inhibitors suggest that this depends entirely on the signalling pathway studied, and possibly on the type of protein-tyrosine kinase that phosphorylates Vav1 in a given signalling complex [19,25]. For example, in BCR-stimulated B cells that signal via Syk, Vav1 activity is not affected by PI3K, whereas in T cells Fyn-mediated tyrosine phosphorylation and PI3K cooperate to activate Vav1 [25]. Regulation of another Vav family member, Vav2, seems to differ slightly. Its activation downstream of EGF receptor stimulation requires PI3K activity whereas tyrosine phosphorylation does not affect its catalytic activity but is instead believed to regulate protein–protein interactions [28]. As for Vav1 though, PI3K-dependent activation of Vav2 does not induce its translocation to the membrane [29]. The regulation of the third family member, Vav3, by PI3K has not been studied yet. However, two recent studies have suggested that, in reverse, Vav3-dependent activation of Rac results in downstream activation of PI3K (see also below) [30,31].

3.2. Sos

Sos is a dual-specificity GEF, with one Ras-specific catalytic domain and another for Rac. In vitro, PtdIns(3,4,5)P_3 has been shown to bind to the PH domain of the DH/PH fragment [19] and to weakly activate Rac-GEF activity in Sos1 immunoprecipitates from COS cells [20]. In vivo, activation of the DH/PH fragment requires PI3K activity [24]. Hence, as with Vav1, PtdIns(3,4,5)P_3 binding to Sos1 is believed to activate its Rac-GEF activity by relieving an intramolecular inhibition [19,24]. In vivo, Sos1 functions as a Rac-GEF downstream of receptor-tyrosine kinases in a complex with the proteins Eps8 and Abi1 [32,33]. Localisation of Sos1 to actin structures is mediated by Eps8 [33], while its Rac-GEF activ-

ity appears to depend on the interaction of the p85 regulatory subunit of type 1A PI3K with Abi1 [20]. The upstream signal for recruitment of p85 to this complex is unclear, it appears to be constitutively associated. Addition of short-chain PtdIns(3,4,5)P_3 to the immunoprecipitated complex circumvents the need for p85 binding to allow Sos1 to exhibit Rac-GEF activity [20], suggesting that the role of p85 binding is to recruit the catalytic PI3K subunit. However, association of actual PI3K activity with the complex and the effect of PtdIns(3,4,5)P_3 in a proper liposome environment remain to be established.

3.3. Tiam

Tiam1 is a Rac-GEF with two PH domains, the C-terminal one being the tandem DH/PH one. Tiam1 membrane association is mainly mediated by the N-terminal PH domain and an adjacent protein/protein interaction domain [34]. Although the N-terminal PH domain binds PtdIns(3,4,5)P_3 on 'fat-blots' [21], Tiam translocation to the membrane does not require PI3K activity [21,35]. The role of PI3K in the activation of Tiam is controversial. The Der lab showed clearly that Tiam1 can be activated by Ras directly and independently of PI3K [36]. Another mechanism of Tiam1 activation is through phosphorylation by Ca^{2+}/calmodulin-dependent kinase II [37]. Confusingly, different studies show either that PI3K is required for Tiam1 Rac-GEF activity in vivo [38], and that PtdIns(3,4,5)P_3 can activate it in vitro (two-fold) [21], or that its activation is PI3K-independent [36]. Unusually for PH domains, the C-terminal Tiam PH domain (in the DH/PH tandem) binds PtdIns3P [39]. PtdIns3P is a PI3K product that can theoretically be made by all types of PI3Ks and is enriched in endosomal and phagosomal membranes [4,40]. Point mutations in the C-terminal PH domain that abolish PtdIns3P binding also abolish the Rac-GEF activity of full-length Tiam1, without affecting its membrane association [41].

3.4. αPIX

αPIX is a GEF that regulates Rac and CDC42 activation downstream of receptor-tyrosine kinases and integrins. Co-expression of membrane-targeted type 1A PI3K with αPIX stimulates its Rac-GEF activity in vivo [22]. Also, lipid vesicles made from dipalmitoyl-PtdIns(3,4,5)P_3, when added to cell culture medium, stimulate Rac-GTP loading in αPIX-transfected but not control cells. Although this method is highly unorthodox, it seems to support the results obtained by overexpression of PI3K [22]. The effects of PtdIns(3,4,5)P_3 on αPIX Rac-GEF activity in vitro have not yet been established, but activation of its CDC42-GEF activity by PtdIns(3,4,5)P_3 is poor [22], suggesting that the effects observed in vivo may not be direct.

3.5. P-Rex1

P-Rex1 is a Rac-specific GEF that is mainly expressed in neutrophils [16]. It is so far the only Rac-GEF purified from tissue to homogeneity on the basis of its PtdIns(3,4,5)P_3-dependent Rac-GEF activity and represents the major such activity in neutrophils. It is substantially, directly and synergistically activated by PtdIns(3,4,5)P_3 and Gβγ subunits both in vitro and in vivo. Its activation in vitro by PtdIns(3,4,5)P_3 is about 20-fold, and it is currently the only Rac-GEF known to be activated directly by Gβγs. P-Rex1 is thus a good candidate to explain Rac activation in a type 1B PI3K-regulated signalling pathway, downstream of G protein-coupled receptors [16]. Mutational analysis is now needed to define the binding sites of PtdIns(3,4,5)P_3 and Gβγs on P-Rex1 and their relative importance for P-Rex1 signalling in vivo.

3.6. SWAP-70

SWAP-70 was originally identified as a protein regulating B cell activation and immunoglobulin class switching. It was not at first recognised to be a GEF, because its PH domain is not preceded by a DH domain. SWAP-70 was purified from a crude bovine brain lysate by fishing with PtdIns(3,4,5)P_3 beads [17]. Only then, careful sequence analysis revealed a weak homology of its C-terminus with DH domains (on the 'wrong' side of the PH domain), and indeed SWAP-70 turned out to be a PtdIns(3,4,5)P_3-dependent Rac-specific GEF in vitro (the level of its activation by PtdIns(3,4,5)P_3 seems comparable to that of P-Rex1). SWAP-70 is involved in signalling downstream of type 1A PI3K-linked membrane receptors in vivo [17].

3.7. Dock180

Dock180 is not a classic GEF, but it is an upstream regulator of Rac that acts in a complex with ELMO [42]. Nucleotide-free Rac can bind directly to a region in Dock180 denoted Docker. In the presence of GTP, this interaction leads to increased Rac-GTP loading, which is why Dock180 has been named a Rac-GEF. However, Dock180 neither has a DH nor PH domain and is unable to bind GDP-bound Rac. Thus, in order for the Dock180/ELMO complex to contribute to Rac activation, there remains a requirement for another enzyme to remove the GDP from Rac. Interestingly, there is a possibility that PI3K might directly regulate Dock180 in some way, as PtdIns(3,4,5)P_3 can bind to a basic region at the C-terminus of Dock180 (that is not a PH domain), albeit without any effect on Rac-GTP loading [43].

PI3K is clearly an important upstream regulator of Rac-GEFs. More in vitro studies are needed, especially with point-mutated full-length GEFs in carefully controlled liposome environments, to better understand the importance of phosphoinositide binding to the PH domain in the DH/PH tandem. Also, more in vivo studies are needed to see how important the input of PI3K is for the activation of Rac-GEFs relative to other regulatory mechanisms in a given signalling pathway.

4. Rac upstream of PI3K

Evidence has been around for many years that Rac can act upstream of PI3K [44,30,31]. The mechanism for this is unclear. In vitro, GTP-bound but not GDP-bound Rac can bind directly to the p85 regulatory subunit of class 1A PI3K [45–47]. The interaction is via the BCR homology domain in p85 and the effector domain of active Rac [45]. One lab has found that this interaction stimulates PI3K in vitro activity [45], whereas another lab found no effect [46]. However, there is no evidence that this is a mechanism by which Rac-mediated PI3K activation can occur in vivo. Recently, the Bourne lab has provided convincing evidence for a positive feedback loop, whereby Rac, once activated by PI3K, can in turn further activate PI3K [48,49]. This positive feedback loop may play a crucial role in establishing cell polarity in neutrophils,

which is a prerequisite for chemotaxis. However, the molecular mechanisms of this feedback loop are still undefined.

5. Future work

The links between PI3K and Rac signalling are clearly essential for the regulation of cellular responses ranging from transcription, translation, protein synthesis, cell survival and cell cycle entry to the structure of the actin cytoskeleton. Future studies must show the relative importance of PI3K for Rac activation compared to other regulatory inputs. Detailed comparisons between different Rac signalling pathways within a cell type and between different cell types are needed to address this. It will also be interesting to see whether PI3K can regulate Rac by other means than via GEFs, e.g. by controlling the inhibition of GAPs or the dissociation of GDIs, both possibilities that have been a little neglected so far. And lastly, the regulatory mechanisms and functional importance of Rac-dependent activation of PI3K clearly need to be studied further.

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