Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1

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Class I phosphoinositide 3-kinases (PI3Ks) are implicated in many cellular responses controlled by receptor tyrosine kinases (RTKs), including actin cytoskeletal remodeling. Within this pathway, Rac is a key downstream target/effecter of PI3K. However, how the signal is routed from PI3K to Rac is unclear. One possible candidate for this function is the Rac-activating complex Eps8–Abi1–Sos-1, which possesses Rac-specific guanine nucleotide exchange factor (GEF) activity. Here, we show that Abi1 (also known as E3b1) recruits PI3K, via p85, into a multimolecular signaling complex that includes Eps8 and Sos-1. The recruitment of p85 to the Eps8–Abi1–Sos-1 complex and phosphatidylinositol 3, 4, 5 phosphate (PIP3), the catalytic product of PI3K, concur to unmask its Rac-GEF activity in vitro. Moreover, they are indispensable for the activation of Rac and Rac-dependent actin remodeling in vivo. On growth factor stimulation, endogenous p85 and Abi1 consistently colocalize into membrane ruffles, and cells lacking p85 fail to support Abi1-dependent Rac activation. Our results define a mechanism whereby propagation of signals, originating from RTKs or Ras and leading to actin reorganization, is controlled by direct physical interaction between PI3K and a Rac-specific GEF complex.

Introduction

Class I phosphoinositide 3-kinases (PI3Ks)* mediate signals emanating from receptor tyrosine kinases (RTKs), and are composed of a catalytic (p110) and a regulatory (p85/p55) subunit (Fruman et al., 1998; Vanhaesebroeck and Waterfield, 1999). Through its SH2 domains, p85 interacts with tyrosine-phosphorylated RTKs, thus recruiting p110 to the plasma membrane, where it can phosphorylate its substrates (Cantrell, 2001). RasGTP also binds to p110 (Rodriguez-Viciana et al., 1994). This is thought to concur to p110 full catalytic activation (Rodriguez-Viciana et al., 1997; Walker et al., 1999). The product of PI3K activity, phosphatidylinositol 3, 4, 5 phosphate (PIP3), is required to regulate a number of Rac-specific guanine nucleotide exchange factors (GEFs), such as Vav, Tiam-1, Swap-70, and Sos-1 (Han et al., 1998; Nimnual et al., 1998; Soisson et al., 1998; Fleming et al., 2000; Shinohara et al., 2002), leading to activation of Rac. These GEFs contain Dbl homology (DH)-pleckstrin homology (PH) domains, which represent the catalytic core and a phosphoinositide-binding surface, respectively. Binding of PIP3 to the PH domain is thought, in the case of Vav and possibly of Sos-1, to relieve an intramolecular inhibitory conformation, thereby allowing catalysis (Han et al., 1998; Nimnual et al., 1998; Das et al., 2000); alternatively, as in the case of Tiam1 and Swap-70, it promotes membrane relocalization (Stam et al., 1997; Shinohara et al., 2002). In a living cell, the situation is likely more complex, as supported by findings that Rac-GEFs assemble into macromolecular complexes that are essential for efficient catalysis and for proper subcellular targeting. An exemplar case is provided by the trimeric complex, Eps8–Abi1–Sos-1 (Scita et al., 1999). This complex is indispensable for the activation of the Rac-specific GEF activity of Sos-1 and for proper localization of PIP3.
the complex to dynamic actin structures (Scita et al., 2001; Innocenti et al., 2002). In addition, disruption of the complex by genetic removal of Eps8 or by dominant-negative Abi1 abrogate Rac activation and Rac-dependent actin remodeling induced by RTKs, Ras, or PI3K (Scita et al., 1999; Innocenti et al., 2002). The present studies were undertaken to understand how the Eps8–Abi1–Sos-1 complex is regulated by upstream signals.

**Results and discussion**

We searched for interaction(s) of the Eps8–Abi1–Sos-1 complex with class I PI3K. Endogenous p85 and Abi1 could be coimmunoprecipitated (Fig. 1 A, left, and supplemental materials), and a wortmannin-sensitive (Fig S1, available at http://www.jcb.org/cgi/content/full/jcb.200206079/DC1) PI3K enzymatic activity was detected in Abi1 immunocomplexes (Fig. 1 A, right), suggesting that the p110–p85 complex binds to Abi1.

GST-p85 or either of its SH2 domains could bind to Abi1 (Fig. 1 B) and to various pTyr-containing proteins, one of which co-migrated with Abi1 (unpublished data). This, and the observation that bacterially produced Abi1 (that is not tyrosine phosphorylated) failed to interact with p85 (Fig S1, D), suggested that the interaction is mediated by pTyr in Abi-1. Indeed, as previously reported, tyrosine phosphorylation of Abi1 was readily detectable (Juang and Hoffmann, 1999; Fan and Goff, 2000) and was abolished by treatment with alkaline phosphatase (Fig. 1 C, left panels and supplemental materials). Finally, a Far-western analysis revealed that the NH2-terminal SH2 of p85 bound directly and specifically to Abi1 in a phosphorylation-dependent manner (Fig. 1 C). Notably, the recruitment of p85 to Abi1 was independent of RTK activation (unpublished data), in agreement with the observation that Abi displays constitutive levels of tyrosine phosphorylation (unpublished data).

Tyr\(^{407}\) of Abi1 was predicted by the NetPhos neural network (Blom et al., 1999) as a strong candidate for phosphorylation by tyrosine kinases (supplemental materials). Mutation of Tyr\(^{407}\) to Phe abrogated the p85–Abi1 interaction both in vitro (Fig 1 D) and in vivo (Fig. 1 E). The overall tyrosine phosphorylation of Abi1Y407F was not reduced, as compared with wild-type Abi1 (unpublished data). Thus, although other tyrosine residues are also phosphorylated in Abi1, Tyr\(^{407}\) is absolutely critical for the interaction with p85. Of note, Tyr\(^{407}\) is present in noncanonical context for ligands of the SH2 domains of p85 (Songyang et al., 1993). Thus, the possibility that in vivo, the interaction between p85 and Abi1 is indirect, cannot be formally excluded, albeit our Far-western data (Fig. 1 C) argue against it.

To assess whether p85 is associated to the Eps8–Abi1–Sos-1 complex (Fig. 2 A), we used Eps8\(^{-/-}\) fibroblasts in which the expression of Eps8 was restored to physiological levels (\(-/-\) [Eps8myc] cells; Scita et al., 2001; Innocenti et al., 2002). Endogenous Sos-1, Abi1, and p85 could be specifically detected in anti-myc immunoprecipitates (Fig. 2 A). In addition, the disruption of the Eps8–Abi1 interaction with the specific PPPPPVYDTEDEE peptide (but not with a control, PPPPPVAAETDEE, peptide; Mongiovı et al., 1999) caused the disappearance of Abi1, p85, and Sos-1 from the anti-myc immunoprecipitates (Fig. 2 A). Previously, we have shown that Eps8, Abi1, and Sos-1 are enriched into membrane ruffles induced by PDGF treatment (Scita et al., 2001). Similarly, endogenous p85 was found to colocalize with Abi1 (Fig. 2 B) and Eps8 (unpublished data) on treatment with PDGF. Thus, p85 is part of an Abi1-based signaling complex that includes Eps8 and Sos-1 in vivo.

The immunoprecipitated Eps8–Abi1–Sos-1 complex displays Rac-specific GEF activity (Scita et al., 1999). This, and

![Figure 1. p85 interacts with Abi1.](http://www.jcb.org/cgi/content/full/jcb.200206079/DC1)
the results presented here, raise the possibility that the recruitment in vivo of PI3K to the complex is also required. Cells were transfected with a combination of Sos-1–mycEps8–Abi1wt or Sos-1–mycEps8–Abi1Y407F, and Rac-GEF assays were performed on anti-myc immunoprecipitates. Trimeric Eps8–Abi1–Sos-1 complexes were readily detected that contained similar amounts of the catalytic subunit Sos-1 (Fig. 3 A). Endogenous p85 (Fig. 3 A) and Rac-GEF activity (Fig. 3 B) were present only in the complexes containing Abi1wt, but not in those with Abi1Y407F.

Next, we performed Rac-GEF assays on Eps8–Abi1wt–Sos-1 or Eps8–Abi1Y407F–Sos-1 immunocomplexes in the presence of water-soluble phosphoinositides. The PH domain of Sos-1 displays selectivity for PI3,4,5P over the more physiologically abundant PI4,5P (Rameh et al., 1997). Consistently, the addition of PI3,4,5P (0.5 μM), the catalytic product of PI3K, but not of PI4,5P, PI5P, or PI3P (unpublished data and Fig. S3), led to a statistically significant increase in the Rac-GEF activity of the Eps8–Abi1wt–Sos-1 complex (Fig. 3 B and supplemental materials). More importantly, it restored the GEF activity of the Eps8–
Abi1Y407F–Sos-1 complex (Fig. 3 B). The two events, p85 recruitment and PIP3 availability, might independently influence the Rac-GEF activity of the tricomplex, or might be somehow integrated. Whatever the case, both the physical presence of p85 and the availability of PIP3 seem to participate in the activation of the Rac-GEF activity of the Eps8–Abi1–Sos-1 complex, at least in vitro.

To rule out the possibility that GEFs other than Sos-1 might be contained in the Eps8–Abi1–Sos-1 complex and be responsible for the observed Rac-GEF activity, we engineered a dominant-negative form of Sos-1, carrying point mutations inactivating the DH domain (Soisson et al., 1998; Mettouchi et al., 2001). Cells were transfected with a combination of mycEps8, Abi1, and either wild-type Sos-1 or Sos-1DH—. Sos-1DH— associates with the Eps8–Abi1 complex as efficiently as wild-type Sos-1 (Fig. 3 C). However, no Rac-GEF activity could be detected in Eps8–Abi1–Sos-1DH— immunocomplexes as compared with the Eps8–Abi1–Sos-1 complex under conditions in which equal amounts of wild-type or mutant Sos-1 were present (Fig. 3, C and D). Moreover, the addition of water-soluble PIP3 increased the Rac-GEF of the Eps8–Abi1–Sos-1, but not of the Eps8–Abi1–SosDH— immunocomplex (Fig. 3 D). Thus, Sos-1 is not only critical for Rac-GEF activity of the Eps8–Abi1–p85–Sos-1 complex, but it is also required to confer responsiveness to PIP3.

The above observations indicate that the recruitment of PI3K by Abi1 into an Eps8–Abi1–Sos-1 complex is necessary and sufficient to activate, in vitro, the Rac-GEF capability of the latter. They further highlight a regulatory role exerted by PIP3 on this complex. If these in vitro findings were to translate into physiologically relevant events, then one would predict that (1) interference with the formation of the Eps8–Abi1–p85–Sos-1 complex either by preventing the binding between Abi1 and p85 (Fig. 3 A) or by genetically removing p85; and (2) pharmacological inhibition of PI3-K by wortmannin should affect Rac activation mediated by the complex in vivo. As show in Fig. 4 A, both the basal and the EGF-induced levels of Rac-GTP were increased by the expression of wild-type Abi1, consistent with the notion that Abi1 is rate-limiting in Rac activation (Innocenti et al., 2002). However, no Rac activity could be detected when the Abi1Y407F, or a mutant of Abi1 (Abi1DY), which does not associate with Eps8, were used (Fig. 4 A). These results strongly suggest that Abi1 mutants, defective in their ability to assemble to PI3K or Eps8, are not only biologically inactive, but act as dominant-negatives, most likely by sequestering the other endogenous components in inactive complexes. Moreover, a formal proof of the requirement of p85 for Abi1-dependent activation of Rac was obtained by using fibroblasts in which both p85 isoforms (α and β) were genetically removed (unpublished data). In these cells, expression of Abi1 failed to induce Rac activation, which was, however, restored by reintroduction of p85α (Fig. 4 B). Finally, treatment with wortmannin reduced (but did not abrogate) EGF-dependent and EGF-independent Rac activation induced by Abi1 (Fig. 4 C). Similarly, the Rac-GEF activity in the Eps8–Abi1–p85–Sos-1 immunocomplex was only reduced by pretreatment of the cells with wortmannin (unpublished data). Thus, all together, these data support the notion that PI3K recruitment to the Eps8–Abi1–Sos-1 complex is physically required to elicit a basal Rac-GEF activity, which is further increased by PIP3.

The trimeric complex Eps8–Abi1–Sos-1 is essential for RTK-mediated actin cytoskeletal remodeling, as witnessed by the lack of PDGF-induced Rac activation and Rac-dependent membrane ruffling detected in Eps8/−/− cells (Scita et al., 1999; Innocenti et al., 2002). To analyze the physiological consequence of p85 recruitment by the tricomplex, two approaches were undertaken. First, a phosphorylated peptide encompassing Tyr<sup>407</sup> of Abi, and corresponding to the Abi1 binding site of p85, was used. This phosphorylated peptide, but not its unphosphorylated version or one in which the tyrosine residue was replaced by phenylalanine, efficiently inhibited the binding of p85 to Abi1, but not to activated PDGFR (Fig. S2). Microinjection of the phosphorylated peptide (but not of the two control peptides) inhibited PDGF-induced ruffles by more than 70% (Fig. 5 A). Notably, inhibition of RTK-mediated ruffles could also be
caused by microinjection of anti-Abi1 antibodies (Scita et al., 1999 and Fig. S4), which, however, did not affect TPA-induced actin remodeling, indicating that additional pathways leading to Rac activation (and possibly reflecting the simultaneous presence of several Rac GEFs in the cells) are at play (supplemental information, Fig. 4). Second, we cotransfected the activated version of either Ras (RasV12) or Rac (RacQL) together with either Abi1wt or the Abi1Y407F mutant, and scored the formation of ruffles. RasV12-induced (but not RacQL-induced) ruffles were efficiently inhibited by coexpression of Abi1Y407F, but not by Abi1wt (Fig. 5 B). Thus, the recruitment of PI3K by Abi1 plays a critical role in RTK-induced actin remodeling and is essential for the propagation of signals from Ras to Rac.

Our results elucidate the molecular mechanisms through which PI3K is coupled to Rac via the Eps8–Abi1–Sos-1 tricomplex. In vitro Rac-GEF assays revealed that the physical interaction with p85 and the availability of PIP3 concur to stimulate the Rac-GEF activity of the tricomplex. This likely reflects the situation in vivo as supported by two observations. First, interference with the association between p85 and the tricomplex results in lack of Rac activation and actin cytoskeleton remodeling. Second, there is an absolute requirement for PIP3 in Rac activation, as shown by several studies using enzymatic inhibitors of PI3K (Cantrell, 2001). The most simple explanation for the sum of our results is that recruitment of p85 to the Eps8–Abi1–Sos-1 tricomplex unMASKS a basal Rac-GEF activity. This is further supported by the finding (Fig. 4) that the overexpression of Abi1 leads to increased Rac-GTP levels, in a p85-dependent manner, even in the absence of growth factor stimulation. However, this basal Rac-GEF activity requires further stimulation by PIP3s, which in vivo, are produced after RTK activation, to achieve a threshold of biological significance. Thus, in vivo, the integrity of the complex and PI3K activity are both necessary for RTK-dependent activation of Rac. However, it is clear that further work will be needed to define the exact interplay between the effects of the physical recruitment of PI3K and of its catalytic activity on the Rac-GEF activity of the Eps8–Abi1–Sos-1 tricomplex. Finally, the RTK-dependent relocalization of the macromolecular complex at sites where membrane ruffling takes place (Fig. 2 B; for review see Scita et al., 2001) suggests that the recruitment of the complex to proper sites within the cell’s formation is key for signal propagation, further ensuring that the production of PIP3 is directed to and modulates the activation of Rac-GEFs, and thereby of Rac, in a defined microenvironment.

Materials and methods
Expression vectors, antibodies, and cells
Cytomegalovirus-, adenovirus-, and elongation factor-1α–promoter-based eukaryotic expression vectors and GST-fusion bacterial expression vectors were generated by recombinant PCR. The mutants of Abi1 (Abi1-Y407F and Abi1-Y283F), in which F replaced the Y residues, and the Sos-1 DH, in...
which the residues E337 and L338 were replaced by A, were generated by PCR-based site-directed mutagenesis and cloned in the expression vectors pCEF1-HA or pCEF1-C1. All constructs were sequence-verified.

Antibodies were as follows: anti-epi85 (Fazioli et al., 1993), anti-Abi1 (Biesova et al., 1997) and anti-EGFR sera (Di Fiore et al., 1990); rabbit polyclonals anti-Sos-1; anti-PDGFr a and β (Santa Cruz Biotechnology, Inc.); monoclonal anti-phosphotyrosine, polyclonal and monoclonal anti-p85 (UBI); anti-v-H-Ras (Oncogene Research Products), anti-HA11 and anti-myc 9E10, (BAbCo); anti-Rac (Transduction Laboratories).

Mouse embryonic fibroblasts (MEFs) were derived from embryos of p85a−/−, p85B−/−, and wild-type mice (129XC57BL6/J). The generation of p85B−/− mice and the establishment of the embryonic fibroblasts from embryos with p85−/− mice were performed as follows: the embryos were transferred onto gelatinized tissue culture dishes and the MEFs were immortalized after few passages using SV-40 large T antigen expressed as retroviral vector. The genotypes of the cells were determined by PCR. Notably, growth factor-mediated activation of Rac, but not of ERK, is completely abrogated in p85 double knockout cells, further supporting the specific and critical role of p85 in this pathway.

**Biochemical assays**

Standard procedure of protein analysis (in vitro binding, cell lysis, coimmunoprecipitation, and Far-western) was as described previously (Fazioli et al., 1993). The lanes labeled “lyase” were loaded with 100 µg of the appropriate lyase.

The levels of Rac-GTP were measured by affinity purification using GST-CRIB (Cdc42 and Rac interactive region) of PAK6 (Manser et al., 1994), as described previously (Scita et al., 1999). Abi1 was coexpressed with wild-type HA-tag Rac at a ratio of 10/1 of Abi1/Rac. Under these conditions, all cells expressing HA-tag Rac were also positive for Abi1. Additionally, the levels of Rac-GTP induced by expression of Abi1 were similar to those obtained by coexpressing HA-Rac with Tiam1, a known GEF for Rac.

In vitro GEF activity toward Rac was performed as described previously (Scita et al., 1999). For the Rac-GEF in Fig. 3 B, performed in the presence of the water-soluble phospholipids, it is noteworthy that PIP3, which restored the Rac-GEF activity of the Ep88–Abi1Y407F–Sos-1 complex (Fig. 3 B), was shown to bind to and permit the association to Rac of the bacterial-produced purified DHR-PH domain of Sos-1 (Das et al., 2000). None of the water-soluble phospholipids used had any effect on the release of guanine nucleotides from [3H]-loaded Rac incubated in the presence of buffer alone or of mock immunoprecipitates. Data are the mean ± SEM of at least three independent experiments performed in triplicate. Results are expressed as the [3H]GDP released after 20 min relative to time 0, and subtracting the background counts released in control reactions (obtained in the presence of immunoprecipitates performed with an ANTI-FLAG® antibody as a control).

**Microinjection of peptides and immunofluorescence**

Peptides were purified by high pressure liquid chromatography (purity grade >95%) and lyophilized (Eurogentec). The peptides solubilized at a concentration of 15 mg/ml in PBS in the presence of 12 mg/ml rabbit IgG were microinjected into quiescent fibroblasts. After 1 h, cells were treated with 10 ng/ml PDGF before fixation and staining.

For the experiments shown in Fig. 5 B, fibroblasts seeded on gelatin were transfected with various combinations of RasV12, RacQL, Abi1WT, and Abi1Y407F as indicated. After serum starvation, cells were fixed and stained with the appropriate antibodies to detect activated Ras or Rac. F-actin was revealed by rhodamine-conjugated phalloidin (red). Detection of ECFP-Abi1WT or ECFP-Abi1Y407F was by epifluorescence (ECFP), In the cotransfection experiments, a molar ratio of 1:3 of the activated GTPases to the ECFP-Abi1 constructs was used so that all the epifluorescent cells also expressed the corresponding GTPase. The percentage of transfected cells (mean ± SEM) undergoing ruffling was as follows: RasV12 = 65 ± 3%; RacV12 + Abi1WT = 62 ± 4%; RasV12 + Abi1Y407F = 16 ± 2%; RacQL = 93 ± 3%; RacQL + Abi1WT = 90 ± 4%; RacQL + Abi1Y407F = 95 ± 5%.

**Mapping of Abi1 tyrosine responsible for the binding to p85**

To map the tyrosine residues of Abi1 responsible for the interaction with p85, we performed a candidate approach. Mutation to phenylalanine of the tyrosine 407 to phenylalanine abrogated the binding of Abi1 to both the NH2-terminal SH2 domain of p85 in

**Online supplemental material**

Fig. S1 shows that (1) the lipid kinase activity associated to Abi1 immunoprecipitates is wortmannin-sensitive; (2) Abi1 and p85 communoprecipitate on overexpression; (3) no interaction could be detected between p85 and bacteriologically produced, GST-fused Abi1 fragments; (4) Abi1 is phosphorylated on tyrosine in mammalian cells; (5) phosphotyrosine 407 (but not 283) of Abi1 mediated the association with the SH2 domain of p85. Fig. S2 shows that the Abi1 peptide encompassing the p85 interaction site phosphorylated on tyrosine 407, but not the one in which Y407 was replaced by F, efficiently competed the p85–Abi1 interaction. Fig. S3 shows the dose-dependent effects exerted by water-soluble PIPS on the Rac-specific GEF activity of the Ep88–Abi1–p85–Sos-1 complex, extending the observations reported in Fig. 3. Fig. S4 shows that microinjection of anti-Abi1 IgG abrogates PDGF-, but not TPA-induced ruffling. Online supplemental material available at http://www.jcb.org/cgi/content/full/jcb.200206079/DC1.

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**References**

Biesova, Z., C. Piccoli, and W.T. Wong. 1997. Isolation and characterization of e3B1, an eps8 binding protein that regulates cell growth. Oncogene. 14:233–241.

Bloom, N., S. Gammeltoft, and S. Brunak. 1999. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J. Mol. Biol. 294:1351–1362.

Cantrell, D.A. 2001. Phosphoinositide 3-kinase signalling pathways. J. Cell Sci. 114:1439–1445.

Das, B., X. Shu, G.J. Day, J. Han, U.M. Krishna, J.R. Falck, and D. Broek. 2000. Control of intramolecular interactions between the pleckstrin homology and DHN homology domains of Vav and Sos-1 regulates Rac binding. J. Biol. Chem. 275:15074–15081.

Di Fiore, P.P., O. Segatto, F. Lonardo, F. Fazioli, J.H. Pierce, and S.A. Aaronson. 1990. The carboxyl-terminal domains of erbB-2 and epidermal growth factor receptor exert different regulatory effects on intrinsic receptor tyrosine kinase function and transforming activity. Mol. Cell. Biol. 10:2749–2756.

Domian, J., R. Dhand, and M.D. Waterfield. 1996. Binding to the platelet-derived growth factor receptor transiently activates the p85α/p110α PI3-kinase-3-kinesin complex in vivo. J. Biol. Chem. 271:21614–21621.

Fan, P.D., and S.P. Goff. 2000. Abi1 interactor 1 binds to sos and inhibits epidermal growth factor receptor and -v-abl-induced activation of extracellular signal-regulated kinases. Mol. Cell. Biol. 20:7591–7601.

Fazioli, F., L. Minichiello, V. Matoska, P. Castagnino, T. Miki, W.T. Wong, and P.P. Di Fiore. 1993. The carboxy-terminus of epidermal growth factor receptor exerts different regulatory effects on intrinsic receptor tyrosine kinase function and transforming activity. J. Biol. Chem. 268:12:3799–3808.

Fleming, L.N., A. Gray, and C.P. Downes. 2000. Regulation of the Rac-specific exchange factor Tiam1 involves both phosphoinositide 3-kinase-dependent and -independent mechanisms. Biochem. J. 351:175–182.

Fruman, D.A., R.E. Meyers, and L.C. Cantley. 1998. Phosphoinositide kinases. Annu. Rev. Biochem. 67:481–507.

Han, J., K. Luby-Phelps, B. Das, X. Shu, Y. Xia, R.D. Mosteller, U.M. Krishna, J.R. Falck, M.A. White, and D. Broek. 1998. Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanine triphos...
phosphatases by Vav. *Science.* 279:558–560.

Innocenti, M., P. Tenca, E. Frittoli, M. Faretta, A. Tocchetti, P.P. Di Fiore, and G. Scita. 2002. Mechanisms through which Sos-1 coordinates the activation of Ras and Rac. *J. Cell Biol.* 156:125–136.

Jiang, J.L., and F.M. Hoffmann. 1999. *Drosophila* abelson interacting protein (dAlb) is a positive regulator of abelson tyrosine kinase activity. *Oncogene.* 18:5138–5147.

Innocenti, M., P. Tenca, E. Frittoli, M. Faretta, A. Tocchetti, P.P. Di Fiore, and G. Scita. 2002. Mechanisms through which Sos-1 coordinates the activation of Ras and Rac. *J. Cell Biol.* 156:125–136.

Juang, J.L., and F.M. Hoffmann. 1999. *Drosophila* abelson interacting protein (dAbi) is a positive regulator of abelson tyrosine kinase activity. *Oncogene.* 18:5138–5147.

Manser, E., T. Leung, H. Salihuddin, Z.S. Zhao, and L. Lim. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature.* 367:40–46.

Mertoussi, A., S. Klein, W. Guo, M. Lopez-Lago, E. Lerniche, J.K. Westrick, and F.G. Giancotti. 2001. Integrin-specific activation of Rac controls progression through the G(1) phase of the cell cycle. *Mol. Cell.* 8:115–127.

Mongiovi, A.M., P.R. Romano, S. Panni, M. Mendoza, W.T. Wong, A. Musacchio, G. Cesareni, and P.P. Di Fiore. 1999. A novel peptide-SH3 interaction. *EMBO J.* 18:5300–5309.

Nimnual, A.S., B.A. Yatsula, and D. Bar-Sagi. 1998. Coupling of Ras and Rac guanosine triphosphatases through the Ras exchanger Sos. *Science.* 279:560–563.

Rameh, L.E., A. Arvidsson, K.L. Carraway III, A.D. Couvillon, G. Rathbun, A. Crompton, B. VanRenterghem, M.P. Czech, K.S. Ravichandran, S.J. Burlauff, D.S. Wang, C.S. Chen, and L.C. Cantley. 1997. A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J. Biol. Chem.* 272:22059–22066.

Rodriguez-Viciana, P., P.H. Warne, R. Dhand, B. Vanhaesebroeck, I. Gout, M.J. Fry, M.D. Waterfield, and J. Downward. 1994. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature.* 370:527–532.

Rodriguez-Viciana, P., P.H. Warne, A. Khwaja, B.M. Marte, D. Pappin, P. Das, M.D. Waterfield, A. Ridley, and J. Downward. 1997. Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. *Cell.* 89:457–467.

Scita, G., J. Nordstrom, R. Carbone, P. Tenca, G. Giardina, S. Gurtkind, M. Bjarnegard, C. Betscholtz, and P.P. Di Fiore. 1999. EPS8 and E3B1 transduce signals from Ras to Rac. *Nature.* 401:290–293.

Scita, G., P. Tenca, L.B. Areces, A. Tocchetti, E. Frittoli, G. Giardina, I. Ponzellini, P. Sini, M. Innocenti, and P.P. Di Fiore. 2001. An effector region in Ep8 is responsible for the activation of the Rac-specific GEF activity of Sos-1 and for the proper localization of the Rac-based actin-polymerizing machine. *J. Cell Biol.* 154:1031–1044.

Shinohara, M., Y. Terada, A. Iwamatsu, A. Shinohara, N. Mochizuki, M. Higuchi, Y. Gotoh, S. Ibara, S. Nagata, H. Itoh, et al. 2002. SWAP-70 is a guanine-nucleotide-exchange factor that mediates signalling of membrane ruffling. *Nature.* 416:759–763.

Soisson, S.M., A.S. Nimnual, M. Uy, D. Bar-Sagi, and J. Kuriyan. 1998. Crystal structure of the Dbl and pleckstrin homology domains from the human Son of sevenless protein. *Cell.* 95:259–268.

Songyang, Z., S.E. Shoreston, M. Chaudhuri, G. Gish, T. Pawson, W.G. Haser, F. King, T. Roberts, S. Rattnek, R.J. Lechleider, et al. 1993. SH2 domains recognize specific phosphopeptide sequences. *Cell.* 72:767–778.

Stam, J.C., E.E. Sander, F. Michels, F.N. van Leeuwen, H.E. Kain, R.A. van der Kamen, and J.G. Collard. 1997. Targeting of Tiam1 to the plasma membrane requires the cooperative function of the N-terminal pleckstrin homology domain and an adjacent protein interaction domain. *J. Biol. Chem.* 272:28447–28454.

Vanhaesebroeck, B., and M.D. Waterfield. 1999. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp. Cell Res.* 253:239–254.

Walker, E.H., O. Perisic, C. Ried, L. Stephens, and R.L. Williams. 1999. Structural insights into phosphoinositide 3-kinase catalysis and signalling. *Nature.* 402:313–320.