Antibodies raised against the 51C/SHIP2 inositol polyphosphate 5'-phosphatase were used to examine the effects of growth factors and insulin on the metabolism of this protein. Immunoblot analysis revealed that the 51C/SHIP2 protein was widely expressed in fibroblast and nonhematopoietic tumor cell lines, unlike the SHIP protein, which was found only in cell lines of hematopoietic origin. The 51C/SHIP2 antiserum precipitated a protein of approximately 145 kDa along with an activity which hydroxylized phosphatidylinositol 3,4,5-trisphosphate to phosphatidylinositol 3,4-bisphosphate. Tyrosine phosphorylation of the 51C/SHIP2 protein occurred in response to treatment of cells with epidermal growth (EGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1), or insulin. EGF and PDGF induced transient tyrosine phosphorylation of 51C/SHIP2, with maximal tyrosine phosphorylation occurring at 5-10 min following treatment and returning to near basal levels within 20 min. In contrast, treatment of cells with NGF, IGF-1, or insulin resulted in prolonged tyrosine phosphorylation of 51C/SHIP2 protein, with 40–80% maximal phosphorylation sustained for up to 2 h following agonist treatment. The kinetics of activation of the Akt/PKB protein kinase by the various factors correlated well with the kinetics of tyrosine phosphorylation of 51C/SHIP2. EGF, NGF, and PDGF stimulated the association of 51C/SHIP2 protein with the Shc adapter protein; however, no Shc could be detected in 51C/SHIP2-immune precipitates from cells treated with IGF-1 or insulin. The data suggest that 51C/SHIP2 may play a significant role in regulation of phosphatidylinositol 3'-kinase signaling by growth factors and insulin.

Activation of phosphatidylinositol 3'-kinase (PI 3-kinase) appears to play a pivotal role in signal transduction by growth factors and insulin (1, 2). PI 3-kinase has the unusual property of phosphorylating phosphatidylinositides on the 3' position generating phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) in vivo (3). These two products are thought to act as second messengers in processes such as mitogenesis (4, 5), oncogenic transformation (6), apoptosis (7), and various types of membrane trafficking (8, 9). Specifically, PtdIns(3,4,5)P3 and PtdIns(3,4)P2 bind to the pleckstrin homology (PH) domains of the Akt/PKB protein kinase and of the PDK1 protein kinase which activates Akt/PKB (10–15). Binding of PtdIns(3,4,5)P3 activates PDK1 and PtdIns(3,4)P2 has been reported to preferentially activate Akt/PKB (12–16). In addition, PtdIns(3,4)P2 and PtdIns(3,4,5)P3 activate several isoforms of protein kinase C (17), and in vitro binding of PtdIns(3,4,5)P3 to the SH2 domains of p85 and Src SH2 has been demonstrated (18).

The production of some forms of phosphoinositides depends on the activities of inositol polyphosphate-5-phosphatases. Many different species of these enzymes exist, with a recently described member, SHIP (for SH2 domain-containing inositol 5-phosphatase), being implicated in receptor signaling in hematopoietic cells (19–21). SHIP is tyrosine phosphorylated in response to treatment of cells with erythropoietin, interleukin-2, interleukin-3, macrophage colony-stimulating factor, B cell receptor cross-linking, and T cell activation (22); however, in most cases, phosphorylation does not appear to affect activity. SHIP has an SH2 domain, multiple proline-rich sites representing possible sites of interaction with SH3 domains, two NPXY motifs, and associates with Shc, Grb2, and SHP-2 under certain conditions (23–25). The 5'-phosphatase activity of SHIP is specific for phosphatidylinositols and inositol which are phosphorylated at the 3' position (23). In some systems, SHIP appears to negatively regulate cell growth (21) or induce apoptosis (25), perhaps counteracting growth factor signals. It is not clear whether tyrosine phosphorylation of SHIP promotes or diminishes these inhibitory effects.

Since PI 3-kinase appears to play a fundamental role in signal transduction in all mammalian cell types and SHIP expression is limited to hematopoietic cells, we examined whether the more widely expressed SHIP-related protein 51C/SHIP2 (26, 27) is involved in signaling from receptor tyrosine kinases in other cells.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Rabbit antiserum were raised against a glutathione S-transferase fusion protein containing carboxyl-terminal residues 1105–1213 of the SHIP2 sequence (27) as described previously (28). Antibody against Shc was from Transduction Laboratories, and anti-phosphotyrosine antibody was from Upstate Biotechnology Inc. Antibody specific for Akt/PKB suitable for immune complex kinase assays was from Upstate Biotechnology Inc. [32P]Protein A, [35S]methionine, and [γ-32P]ATP were from Amersham Pharmacia Biotech Corp. SHIP antiserum was a kind gift from M. Kavanaugh, Chiron Corp. Cell Culture—SH-SY5Y human neuroblastoma cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. PC12 cells (a kind gift from D. Maysinger, McGill University) were grown in Dulbecco’s modified Eagle’s medium with 10% horse serum and 5% fetal bovine serum. The 3T3-L1 preadipocyte cell line was maintained in Dulbecco’s modified Eagle’s medium with 10% calf serum and induced to differentiate to adipocyte-like cells following the protocol of Rubin et
Tyrosine Phosphorylation of the 51C/SHIP2 Protein

Immune Precipitation—Cells were cultured in 100-mm dishes, treated with agonist for the indicated times, washed twice with 10 ml of cold phosphate-buffered saline, and lysed in 1 ml of buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EGTA, 10 mM sodium pyrophosphate, 10 mM NaF, 30 mM Na-orthovanadate, 10 mM aprotinin, 10 μM leupeptin, and 1 mM benzamidine (modified HNTG buffer as described by Margolis et al. (30)). Extracts were centrifuged for 10 min at 10,000 x g, and supernatants were incubated for 60 min at 4 °C with the desired antisera. Immune complexes were collected on protein A-Sepharose beads, washed three times with 1 ml of lysis buffer, and analyzed on SDS-polyacrylamide gels as described previously (28).

Immunoblotting—For analysis of whole cell levels of 51C/SHIP2 and SHIP, cells were grown in 35-mm dishes, washed with phosphate-buffered saline, and lysed in SDS sample buffer. Samples containing 25 μg of protein were loaded onto 4–15% polyacrylamide-SDS gels and transferred to nitrocellulose membranes, and the membranes were probed with preimmune serum, 51C/SHIP2 antisera, or SHIP antisera. For other experiments, immunoprecipitated proteins were resolved on SDS-gels, transferred to nitrocellulose, and probed with anti-phosphotyrosine antibody (4G10, Upstate Biotechnology) (1:100 dilution in 2% bovine serum albumin) or with a 1:1000 dilution of Shc phosphotyrosine antibody (4G10, Upstate Biotechnology) (1:1000 dilution in PBS). Immune complexes were bound to protein A-Sepharose beads for 3 h at 4 °C. Immune precipitates were washed three times with 25 mM HEPES, pH 7.8, 10 mM NaCl, 1% Triton X-100, 30 mM sodium pyrophosphate, 10 mM NaF, 10 mM EDTA, 1 mM dithiothreitol, and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM NaVO4). Lysates were cleared and immunoprecipitated with 4 μg of Akt antibody (Upstate Biotechnology No. 60-608) bound to protein A beads for 3 h at 4 °C. Immune precipitates were washed three times with 25 mM HEPES, pH 7.8, 10 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreitol and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% bovine serum albumin, and 1% dithiothreito...
Tyrosine Phosphorylation of the 51C/SHIP2 Protein

FIG. 2. 51C/SHIP2 immunoprecipitates contain PtdIns(3,4,5)P3-phosphatase activity. Approximately 2 x 10^7 SH-SY5Y cells which had been treated with vehicle (Con) or with 25 ng IGF-1 (IGF-1) for 10 min were lysed and precipitated with preimmune serum or with 51C/SHIP2 antisera as described under "Experimental Procedures." PtdIns(3,4,5)P3 labeled at the 3'-position with [32P]orthophosphate was added to the immunoprecipitates and incubated for 20 min at room temperature (during which time product formation was linear). Samples were extracted and separated by TLC. The positions of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 standards are indicated. The data are representative of three separate experiments.

FIG. 3. Effects of growth factors and insulin on tyrosine phosphorylation of the 51C/SHIP2 protein. SH-SY5Y cells were treated with vehicle (C), 25 ng EGF (E), 25 ng PDGF (P), or with 25 ng IGF-1 (I); PC-12 cells were treated with vehicle (C) or with 25 ng NGF (N); and 3T3-L1 adipocytes with vehicle (C) or 25 ng insulin (Ins) for 5 min prior to lysis and immunoprecipitation as described under "Experimental Procedures." Lysates were immunoprecipitated with 51C/SHIP2 anti-serum, except for the first SH-SY5Y lane and the first Rous sarcoma virus (RSV) lane, where preimmune serum was added. Following immunoprecipitation tyrosine phosphorylation of proteins was analyzed by immunoblotting with anti-phosphotyrosine antibodies as described under "Experimental Procedures." The 145-kDa position on the blots is indicated (145). The data are representative of three separate experiments.

Tyrosine phosphorylation could regulate the activity of 51C/SHIP2 leading to changes in intracellular levels of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 which could influence the activity of the Akt/PKB protein kinase (12–16). For this reason, the effects of the various growth factors on the extent and duration of Akt/PKB activation were determined. (Fig. 5). EGFR stimulated a transient activation of Akt/PKB which returned to near baseline levels after 20 min of treatment. IGF-1 and insulin treatment resulted in a somewhat higher maximal level of activation which was sustained for at least 2 h. NGF and PDGF treatment produced equivalent maximal activation of Akt/PKB relative to IGF-1 and insulin but of shorter duration.

The 52- and 66-kDa tyrosine-phosphorylated proteins co-precipitating with 51C/SHIP2 were similar in size to two forms of Shc, and the 52-kDa form of Shc has been shown to associate with SHIP, leading us to directly investigate whether the co-precipitating proteins were forms of Shc. In these experiments, lysates from cells treated with growth factors and insulin were immune-precipitated with 51C/SHIP2 antisera and immunoblotted with Shc antibodies (Fig. 6). The 52- and 66-kDa forms of Shc co-precipitated with 51C/SHIP2 protein from SH-SY5Y cells treated with EGF and PDGF, and the 52-kDa form co-precipitated with 51C/SHIP2 from NGF-treated PC-12 cells. No Shc was detected in 51C/SHIP2 immunoprecipitates from insulin-treated 3T3-L1 cells or IGF-1-treated SH-SY5Y cells. Even though the 66-kDa form of Shc was equally abundant in SH-SY5Y cells and PC-12 cells, it associated with 51C/SHIP2 from EGF- and PDGF-treated SH-SY5Y cells, but not with 51C/SHIP2 from NGF-treated PC-12 cells.

DISCUSSION

The 51C/SHIP2 protein is a prominent substrate for tyrosine phosphorylation in response to treatment of cells with a number of growth factors and insulin. These results suggest that 51C/SHIP2 may be a general modulator the PI 3-kinase response to growth factors and insulin by regulating levels of PtdIns(3,4,5)P3 and PtdIns(3,4)P2. As with SHIP, the question arises whether 51C/SHIP2 activity would function to positively or negatively regulate growth factor signaling. For example, the PH domain of Akt/PKB binds both PtdIns(3,4,5)P3 and PtdIns(3,4)P2, however, only binding of PtdIns(3,4,5)P3 has been reported to result in activation of the enzyme (11, 15, 16). On the other hand, PDK1, which phosphorylates and partially activates Akt/PKB, is more strongly activated by PtdIns(3,4,5)P3 than by PtdIns(3,4)P2 (13, 14). If 51C/SHIP2 is activated by growth factors and plays a positive role in signaling, it could increase levels of PtdIns(3,4,5)P3 without totally depleting PtdIns(3,4)P2 and bring about increased activation of Akt/PKB or other downstream effectors of PI 3-kinase. Alternatively, 51C/SHIP2 activity could function to deplete PtdIns(3,4,5)P3 levels and attenuate PI 3-kinase signaling and the effect of growth factors would be to inhibit 51C/SHIP2 activity and potentiate PI 3-kinase signaling. In support of the latter hypothesis, overexpression of SHIP in the FD hematopoietic cell line inhibited mitogenic signaling by macrophage colony-stimulating factor (22) and microinjection SHIP into Xenopus oocytes inhibited insulin-induced germinal vesicle breakdown (34).

The role to tyrosine phosphorylation of SHIP and 51C/SHIP2 in the function of these proteins is not well understood. Osborne et al. (24) have shown that tyrosine phosphorylation of SHIP by Lck inhibits SHIP activity, suggesting that SHIP is normally active and that one effect of agonist treatment would be to inhibit its activity in order to increase PI 3-kinase-generated levels of PtdIns(3,4,5)P3. Constitutive phosphorylation of SHIP in bcr-Abl-transformed cells and of 51C/SHIP2 in Rous sarcoma virus-transformed cells could support this model, since transformation by these oncogenes activates PI 3-kinase and results in elevated basal levels of PtdIns(3,4,5)P3 (35, 36). Our data showing prolonged tyrosine phosphorylation of 51C/SHIP2 induced by IGF-1, NGF, and insulin could also support this model. IGF-1 and NGF are important survival factors which protect cells from apoptosis, and activation of Akt/PKB through the PI 3-kinase appears to represent a significant pathway in the anti-apoptotic activity of these growth factors (7, 32, 37). Insulin signaling has also been shown to be dependent on PI 3-kinase signaling through Akt/PKB, which has been shown to be involved in both GLUT4 translocation (9) and activation of glycogen synthesis (38). Inhibition of PtdIns(3,4,5)P3 hydrolysis to PtdIns(3,4)P2 by relatively long term tyrosine phosphorylation of 51C/SHIP2 could yield ratios of these mediators, which would potentiate PI 3-kinase signal-
ing and contribute to the prolonged activation of Akt/PKB seen with IGF-1 and insulin. However, thrombin stimulation of platelets leads to translocation of SHIP and PI 3-kinase to the actin cytoskeleton, coincident with prolonged tyrosine phosphorylation of SHIP and with the accumulation of PtdIns(3,4)P₂ (39, 40). In this case, agonist-stimulated tyrosine phosphorylation of SHIP appears to increase its activity (i.e. increase generation of PtdIns(3,4)P₂) and SHIP appears to play a positive role in thrombin signaling. The transient tyrosine phosphorylation of Shc/SHIP2 by EGF and PDGF may correlate with a shorter duration of activation of PI 3-kinase by these factors in the cell types we have examined.

Another point of divergence for signaling by the different growth factor receptors could be the ability to induce association of Shc/SHIP2 with Shc. EGF, PDGF, and NGF clearly stimulate the association of Shc/SHIP2 with Shc. On the other hand, IGF-1 and insulin stimulate comparable tyrosine phosphorylation of Shc/SHIP2 relative to EGF and NGF, but fail to promote formation of Shc/SHIP2-Shc complexes. The binding of Shc to Shc/SHIP2 and SHIP may serve to attenuate signaling through the Ras pathway by competing with Grb2/Sos complexes (41). The differences in Shc binding to Shc/SHIP2 found for EGF, PDGF, and NGF versus insulin and IGF-1 may reflect the relative importance of the Ras pathway in cellular responses generated by these growth factors. These differences in interaction with Shc may also influence the capacity of the different factors to stimulate Akt/PKB activity.

In addition, the 66-kDa form of Shc associated with Shc/SHIP2 from EGF- and PDGF-treated SH-SY5Y cells, but not from NGF-treated PC-12 cells, even though similar levels of 66-kDa Shc were present and tyrosine phosphorylated by respective agonists in both cell types. This form of Shc has recently been reported to limit activation of mitogen-activated protein kinase by EGF (42), perhaps by competing Grb2/Sos complexes away from the 52-kDa form of Shc. The capacity to stimulate interaction of 66-kDa Shc with Shc/SHIP2 could contribute to differences in the kinetics of activation of mito-

**FIG. 4.** Time courses of phosphorylation of the Shc/SHIP2 protein in response to growth factors and insulin. SH-SY5Y cells were treated for the indicated times with 25 nM EGF, 25 nM PDGF, or 25 nM IGF-1. PC-12 cells and 3T3-L1 adipocytes were treated with 25 nM NGF or 25 nM insulin, respectively, for the indicated times. Following treatment, cells were lysed and immunoprecipitated with Shc/SHIP2 antiserum as described under “Experimental Procedures.” Immunoblot analysis with anti-phosphotyrosine antibody was performed as in Fig. 3. The data are representative of two experiments with very similar results.

**FIG. 5.** The time course of stimulation of Akt/PKB activity in response to growth factor and insulin treatment. SH-SY5Y cells were treated for the indicated times with 25 nM EGF, 25 nM PDGF, or 25 nM IGF-1. PC-12 cells and 3T3-L1 adipocytes were treated with 25 nM NGF or 25 nM insulin, respectively, for the indicated times. Following treatment, cells were washed with phosphate-buffered saline, and lysates were prepared for assay of Akt/PKB activity as described under “Experimental Procedures.” In a representative experiment, maximal counts/min incorporated into GSK-3 peptide were 6845 cpm for EGF, 16875 cpm for PDGF, 21765 cpm for IGF-1, 19875 cpm for NGF, and 22734 cpm for insulin. Similar amounts of Akt/PKB were immunoprecipitated from each lysate (not shown). The data are representative of two experiments with similar results.

**FIG. 6.** Growth factor-stimulated association of Shc with Shc/SHIP2. SH-SY5Y cells were treated for 5 min with vehicle (C), 25 nM EGF (E), 25 nM PDGF (P), or 25 nM IGF-1 (I); PC-12 cells were treated for 5 min with vehicle (C) or 25 nM NGF (N); and 3T3-L1 adipocytes were treated for 5 min with vehicle (C) or 25 nM insulin (Ins). Cells were lysed and immunoprecipitated with Shc/SHIP2 antiserum as described under “Experimental Procedures” prior to immunoblot analysis with anti-Shc. In lanes marked S, whole cells lysates from each cell type were resolved on SDS-gels and immunoblotted for Shc expression. The data are representative of three experiments which gave similar results.
Tyrosine Phosphorylation of the 51C/SHIP2 Protein

18609

gen-activated protein kinase observed for EGF versus NGF (4).
In summary, the 51C/SHIP2 protein is a novel substrate
tyrosine phosphorylated in response to treatment of cells with
growth factors and insulin and which may serve varied func-
tions for different growth factors and cell types. Future efforts
will be directed toward more detailed delineation of the pro-
cesses regulated by 51C/SHIP2.

Acknowledgments—We thank Mike Kavanaugh for generously pro-
viding SHIP antiserum and Heidi Camp and Mathew Brady for help
with 3T3-L1 cells.

REFERENCES

1. Vanhaesebroeck, B., Stein, R., and Waterfield, M. D. (1996) Cell. Signalling
27, 249–270

2. Toker, A., and Cantley, L. C. (1997) Science 278, 673–676

3. Whitman, M., Downes, C. P., Keeler, T., and Cantley, L. C. (1988) Nature
332, 644–646

4. Marshall, C. J. (1995) Cell 80, 179–185

5. Toker, A., and Cantley, L. C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1689–1693

6. Liu, L., Damen, J. E., Ware, M. D., and Krystal G. (1997) J. Biol. Chem. 272, 10998–11001

7. Pesesse, X., Deleu, D., De Smedt, F., and Erneux, C. (1997) Biochem. Biophys. Res. Commun. 239, 285–287

8. Han, S., Downes, C. P., and Cantley, L. C. (1994) J. Biol. Chem. 269, 26857–26863

9. Okada, T., Kawano, T., Sakakibara, T, Hazeaki, O., and U, M. (1994) J. Biol.
Chem. 269, 3568–3573

10. Franke, T. F., Yang, S. I., Chan, T. O., Datta, K., Kazlauskas, A., Morrison,
D. K., Kaplan, D. R., and Tsichlis, P. N. (1995) Cell 81, 727–736

11. Klippel, A., Kavanaugh, W. M., Pot, D., and Williams, L. T. (1997) Science 276, 1848–1850

12. Alessi, D. R., James, S. R., Downes, C. P., Keeler, T., and Cantley, L. C. (1988)
Science 240, 785–789

13. Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., and Cohen,
P. (1997) Curr. Biol. 7, 776–779

14. Alessi, D. R., Deak, M., Casamayor, A., Casadivel, F. B., Morris, N., Norman,
D. G., Gaffney, P., Reese, C. B., MacDougall, C. N., Habison, D., Ashworth,
A., and Bownes, M. (1997) Curr. Biol. 7, 776–779

15. Franke, T. F., Kaplan, D. R., Cantley, L. C., and Toker, A. (1997) Science 275, 665–668

16. Frech, M., Andjelkovic, M., Ingle, E., Reddy, K. K., Falck, J. R., and Hem-
ings, B. A. (1997) J. Biol. Chem. 272, 5474–2481

17. Toker, A., Meyer, M., Reddy, K. K., Falck, J. R., Anjea, R., Aneja, S., Parras, A.,
Burns, D. J., Ballas, L. M., and Cantley, L. C. (1994) J. Biol. Chem. 269, 32358–32367

18. Rameh, L., Chen, C.-S., and Cantley, L. C. (1995) Cell 83, 821–830

19. Damen, J. E., Lint, L., Rosten, P., Humphries, R. K., Jefferson, A. B., Majerus,
P. W., and Krystal G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1689–1693

20. Kavanaugh, W. M., Pot, D. A., Chin, S. M., Deuter-Reinhard, M., Jefferson,
A. B., Norris, F. A., Masiarz, F. R., Censous, L. S., Majerus, P. W., and
Williams, L. T. (1996) Curr. Biol. 6, 438–445

21. Liu, L., Damen, J. E., Saito, H., Merkens, L. S., Tittle, T. V., Jakobs, R. M., Whitney,
M. A., Grompe, M., Friedman, A. S., and Moses, R. E. (1996) Gomemics 29,
285–287

22. Liu, L., Damen, J. E., Hughes, M. R., Babic, I., Jirik, F. R., and Krystal, G.
(1997) J. Biol. Chem. 272, 8983–8988

23. Hejna, J. A., Saith, H., Merkens, L. S., Tittle, T. V., Jakobs, R. M., Whitney,
M. A., Grompe, M., Friedman, A. S., and Moses, R. E. (1996) Biochimia
249–270