The nonreceptor tyrosine kinase Fer and the closely related protein, Fes, are the only members of a group of proteins that is unrelated to any other families of cytoplasmic tyrosine kinases (1). The 94-kDa isoform of Fer and Fes share the same conserved domain organization, which consists of a long N-terminal domain, a central SH2 domain, and a C-terminal kinase domain. Contained within the N-terminal domain are three coiled-coil domains, which have been shown to be required for oligomerization and have been shown to trimerize in vivo (2, 3). The extreme N terminus of Fer also contains a Fes/CIP4 homology domain, which has also been found in proteins involved in regulating the actin cytoskeleton, and may function in microtubule binding (4, 5). Fer is ubiquitously expressed in a wide number of cell types and tissues (6, 7).

In a screen for 3T3-F44A adipocyte proteins that bind SH2 domains, we isolated a cDNA encoding Fer, a non-receptor protein-tyrosine kinase of the Fes/Fps family that contains a functional SH2 domain. A truncated splicing variant, iFer, was also cloned. iFer is devoid of both the tyrosine kinase domain and a functional SH2 domain but displays a unique 42-residue C terminus and retains the ability to form oligomers with Fer. Expression of both Fer and iFer proteins are strikingly increased upon differentiation of 3T3-L1 fibroblasts to adipocytes. Platelet-derived growth factor treatment of the cultured adipocytes caused rapid tyrosine phosphorylation of Fer and its recruitment to complexes containing platelet-derived growth factor receptor and the p85 regulatory subunit of phosphatidylinositol (PI) 3-kinase. Insulin treatment of 3T3-L1 adipocytes stimulated association of Fer with complexes containing tyrosine phosphorylated IRS-1 and PI 3-kinase but did not stimulate tyrosine phosphorylation of Fer. PI 3-kinase activity in anti-Fer immunoprecipitates was also acutely activated by insulin treatment of cultured adipocytes. These data demonstrate the presence of Fer tyrosine kinase in insulin signaling complexes, suggesting a role of Fer in insulin action.

The protein-tyrosine kinase Fer associates with signaling complexes containing insulin receptor substrate-1 and phosphatidylinositol 3-kinase.*

Through both subcellular fractionation and immunocytochemistry, Fer has been shown to be localized to the cytoplasm and the nucleus (8).

Although the cellular function of Fer is not known, its tyrosine phosphorylation has been shown in A431 and Swiss 3T3 fibroblasts following activation of the epidermal growth factor receptor or the PDGF receptor (3). PDGF stimulation also results in an increase in Fer tyrosine kinase activity. These results indicate that Fer may function in signaling by growth factor receptors. One potential role Fer may have in signaling is in the regulation of cellular adhesion complexes. It has been previously demonstrated that the N-terminal region of Fer is constitutively bound to a cell adhesion complex protein p120cas, which itself is tyrosine phosphorylated upon growth factor stimulation (3). Fer also co-immunoprecipitates with β-catenin, another component of cell adhesion complexes. Overexpression of Fer in embryonic fibroblasts elevates tyrosine phosphorylation of both p120cas and β-catenin and decreases the amount of α-catenin and β-catenin that associates with E-cadherin. This indicates that Fer overexpression results in the dissolution of adherens junction complexes (9).

Recently the SH2 domain of Fer has been also shown to bind to the F-actin-binding protein cortactin. A dominant negative mutant in Fer inhibits the ability of macrophage colony stimulating factor to stimulate tyrosine phosphorylation of cortactin (10). Thus it is possible that Fer may also link growth factor signaling to cytoskeletal elements.

During a screen of SH2-binding proteins using a 3T3-F44A adipocyte expression library, we identified a large number of clones consisting of the C-terminal kinase domain of Fer. In these differentiated adipocyte cells, insulin has the unique ability to activate a number of metabolic pathways such as glucose uptake (11). This led us to speculate that Fer might possibly also have a role in insulin signaling. In the present study we show that Fer expression increases upon 3T3-L1 adipocyte differentiation and that these cells express a splicing isoform that lacks most of the SH2 domain and all of the C-terminal kinase domain. We also show that insulin stimulation results in the formation of complexes between Fer with tyrosine phosphorylated IRS-1 and p85 phosphatidylinositol kinase.

**EXPERIMENTAL PROCEDURES**

**Materials—** 4G10 anti-phosphotyrosine monoclonal antibody and monoclonal p85 (N-SH2) antibody used for immunoblotting or immuno-
noprecipitation were purchased from Upstate Biotechnology Inc. Rabbit polyclonal IRS-1 (SC559) and PDGF receptor antibody was purchased from Santa Cruz Biotechnology. Mouse monoclonal antibody HA antibody (12CA5) was purchased from BABCOC. Rabbit anti-HA antibody was raised against a peptide corresponding to the HA epitope and purified under conditions that separate the HA epitope from NaF, 1 mM EDTA, and then precipitated with a peptide containing the last 15 C-terminal amino acids of iFer.

**Cell Culture**—3T3-L1 fibroblasts (2–3 days post confluent) were differentiated into adipocytes by incubating with DMEM containing 25 mM glucose, 10% fetal bovine serum, 50 μg/mL streptomycin, 50 units/mL penicillin, 0.5 mM isobutylmethylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin for 3 days and then grown in DMEM with 10% Fetal bovine serum, 50 μg/mL streptomycin and then incubated additional 5–8 days. COS-1 cells were grown in DMEM containing 10% fetal bovine serum, 50 μg/mL streptomycin, and 50 units/mL penicillin.

**Expression Cloning of Grb2 Binding Proteins**—A λ-Zap mouse 3T3-F442A was screened for Grb2-binding proteins using the method described in Singh et al. (12). Briefly, 40,000 plaque forming units of this library were plated on to eighteen 15-cm LB plates in LB top agar and then incubated for an additional 10 h at 37 °C. Plates were cooled to 4 °C, after which filters were removed and washed three times in binding buffer (30 mM Hepes, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol) under constant agitation. Filters were then blocked with 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 5% nonfat milk and washed several times in binding buffer. GST-Grb2 fusion protein was produced from a pGEX-2TK construction (13). GST-Grb2 fusion protein was 32P-labeled by incubating fusion protein with [γ-32P]ATP (100 μL of 100 μM reduced glutathione and then incubated with 100 mM Tris-HCl, pH 7.5, and 500 mM LiCl), one time with wash buffer 2 (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA) and one time with reaction buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.5 mM EDTA, 10 mM MgCl2, and 120 μM adenosine). Sepharose-bound immune complexes were then incubated for 10 min at 30 °C with 40 μL of reaction buffer with 100 μM phosphatidylinositol, 100 μM phosphatidylycerine, and 10 μM [γ-32P]ATP. Reactions were quenched with HCl, and extracted with methanol:chloroform (1:1). Labeled phospholipids were spotted onto PE SIL G plates, and phosphatidylinositol monophosphates were separated by borate thin layer chromatography.

**RESULTS**

**Cloning of the iFer Splicing Variation**—Through screening a 3T3-F44A adipocyte expression library for proteins that bind to SH2 domain of Grb2, we isolated several clones consisting of the C-terminal portion of Fer including its tyrosine kinase. To determine which isoforms of Fer are expressed in this cell line, we screened this library with 32P-labeled DNA probes consisting of a portion of the Fer coding sequence. Six independent clones that hybridized to this probe were isolated. Four of these clones, which we designated as iFer, contained the entire Fer coding sequence except for a 65-base pair deletion within the region that encodes for the SH2 domain. This deleted region begins with the sequence GT, which is consistent with it being the beginning of an alternately spliced intron. The iFer splicing deletion results in a nonsense mutation which encodes a 60-kDa protein with a partial SH2 domain and a unique C-terminal region (Fig. 1).

**Fer and iFer Can Oligomerize in Vivo**—Both Fer and iFer were tagged with HA epitopes and expressed in COS-1 cells to determine whether they encode active kinases. Consistent with previously published results, Fig. 2A shows that overexpressed 94-kDa Fer in COS-1 cells is constitutively active and becomes autophosphorylated on tyrosine residues. The protein encoded by the iFer splicing variant lacks a functional tyrosine kinase domain but contains one of three major Fer tyrosine phosphorylation sites (2). As seen in Fig. 2A, iFer fails to get tyrosine phosphorylated when overexpressed in COS cells, indicating that a functional Fer kinase domain is required for this phosphorylation to occur. Because the N-terminal region of Fer contains the domain that is required for its oligomerization, we reasoned that iFer is still capable of forming complexes with Fer. To test this, we overexpressed HA-tagged iFer, along with...
Fer that is tagged with both the Myc and HA epitope, and immunoprecipitated Fer oligomers with anti-Myc antibodies. Fig. 2B shows that iFer can co-immunoprecipitate with Fer. Furthermore co-expression of Fer and iFer also results in the tyrosine phosphorylation of iFer, which strongly implicates that its tyrosine phosphorylation site can be phosphorylated in trans by an active Fer kinase.

Both Fer and iFer Are Highly Expressed upon 3T3-L1 Adipocyte Differentiation—Fer has been shown to be ubiquitously expressed in most tissues and cell lines that have been studied. To confirm that Fer is also expressed in differentiated adipocytes, total cell lysates from both 3T3-L1 fibroblasts and adipocytes were separated on SDS-PAGE, transferred to PVDF filters, and blotted with anti-Fer antibodies. As seen in Fig. 3A, Fer is expressed in both adipocytes and preadipocytes, but its expression is induced approximately 5-fold by differentiation.

To substantiate that iFer is also expressed in these cell lines, we raised antibodies to a peptide composed of the last 15 amino acids of iFer.
FIG. 2. Tyrosine phosphorylation of Fer and oligomerization of Fer with iFer. A, COS-1 cells were transfected with either 3X HA Fer or iFer DNA. Two days after transfection, cell lysates were immunoprecipitated with anti-HA polyclonal antibody. Immunoprecipitated proteins were separated on 7.5% SDS-PAGE, transferred to nitrocellulose, and immunoblotted with either anti-HA monoclonal antibody 12CA5 (left panel) or phosphotyrosine (4G10) antibody (right panel). The migration of Fer and iFer is indicated by the arrows. B, COS-1 cells were transfected with Myc-HA-Fer, HA-iFer, or both constructs. Cells were lysed and immunoprecipitated with anti-Myc monoclonal antibody (10E2). Immunoprecipitated proteins were separated on 7.5% SDS-PAGE and transferred to PVDF membranes. Membranes were immunoblotted with either anti-HA monoclonal antibody 12CA5 (top panel) or phosphotyrosine (4G10) antibody (bottom panel). IP, immunoprecipitation; PY, phosphotyrosine.

FIG. 3. Expression of Fer and iFer in 3T3-L1 adipocytes. Triton-soluble protein lysates were prepared from 3T3-L1 adipocytes that were serum-starved or stimulated with insulin for 10 min. Lysates (50 μg) were separated on 7.5% SDS-PAGE and transferred to PVDF membranes. A, membranes were immunoblotted with anti-FerK serum. B, membranes were blotted with either anti-iFer antibody (left panel) or preimmune serum (right panel).

acids of the predicted iFer coding sequence. This sequence does not have any significant homology to any proteins currently deposited in nonredundant protein sequence data bases. Western blot analysis revealed a band of approximately 72 kDa that is expressed in 3T3-L1 adipocytes but not in fibroblast hybridizes to anti-iFer antiserum (Fig. 2B). This band is not recognized by preimmune serum. The difference in the mobility of this band to that of the predicted molecular mass of iFer based on its sequence may be due to post-translational modifications, and insulin stimulation does not effect the mobility of this band on SDS-PAGE.

PDGF Stimulates Tyrosine Phosphorylation of Fer and Complex Formation between Fer, PDGF Receptor, and p85—In mouse Swiss 3T3 fibroblast cell lines, PDGF stimulates tyrosine phosphorylation of Fer and complex formation of Fer with activated PDGF receptors (3). Because 3T3-L1 adipocytes are highly responsive to PDGF stimulation, we were interested in ascertaining whether a similar response to this growth factor occurs in this cell type (16). To determine this, we stimulated serum-starved 3T3-L1 adipocytes with PDGF for 5 min and immunoprecipitated cell lysates with anti-Fer antibody. These complexes were separated on SDS-PAGE and immunoblotted with anti-Fer and anti-phosphotyrosine antibodies. Fig. 4 shows that similar to Swiss 3T3-L1 fibroblasts, PDGF stimulation of 3T3-L1 adipocytes results in an increase of Fer tyrosine phosphorylation (top panel). Furthermore, Fer also co-immunoprecipitates with tyrosine phosphorylated PDGF receptor. PDGF stimulation also results in the recruitment of phosphatidylinositol 3-kinase to tyrosine phosphorylated PDGF receptor. Thus PDGF stimulation should produce PDGF receptor complexes containing Fer and the p85 subunit of phosphatidylinositol 3-kinase (15). Immunoblotting of anti-Fer immunoprecipitates reveals that p85 does in fact bind to complexes containing Fer in a PDGF-dependent manner (Fig. 4, middle panel). Only a small amount of p85 binds to Fer in the absence of PDGF stimulation.

Fer Is Bound to Tyrosine Phosphorylated IRS-1 and p85 Subunit of Phosphatidylinositol 3-Kinase in Response to Insulin—In 3T3-L1 adipocytes, many genes required for insulin signaling pathways, such as insulin receptor and GLUT-4, become highly expressed upon differentiation (17–19). Because Fer is also highly expressed upon differentiation, we reasoned that it may also have a role in insulin signaling. To test this possibility, we stimulated serum-starved 3T3-L1 adipocytes with insulin for 5 min and immunoprecipitated lysates with anti-Fer antibody. Unlike PDGF stimulation of cultured adipocytes, insulin treatment results in little if any increase in tyrosine phosphorylation of Fer (Fig. 5, middle panel). However, insulin stimulation does result in a marked increase in complex formation between Fer and tyrosine phosphorylated IRS-1. Similar to PDGF, insulin stimulation also promotes complex formation between Fer and p85 (Fig. 5, bottom panel). We also blotted these immunoprecipitates for the presence of Grb2, but we were unable to detect Grb2 (data not shown). This
indicates that the original finding of Fer in a screen for proteins that bind to the Grb2 SH2 domain is probably due to the ability of the kinase domain of Fer to catalyze tyrosine phosphorylation of Grb2 binding positive plaques.

The Binding of Fer to Tyrosine Phosphorylated IRS-1 and Phosphatidylinositol 3-Kinase Is an Early Event of Insulin Activation—
The time course of the formation of Fer/IRS-1 and Fer/p85 complexes was determined by immunoprecipitating Fer from 3T3-L1 adipocytes stimulated with insulin for various times. Fig. 6 shows that within 30 s of insulin stimulation Fer is maximally bound to both tyrosine phosphorylated IRS-1 and p85. The binding of Fer to both these molecules is fully sustained 1 h after initial insulin stimulation. To determine whether active phosphatidylinositol 3-kinase is associated with Fer, immunoprecipitates were assayed for their ability to label phosphatidylinositol with $[^{32}\text{P}]ATP$. As seen in Fig. 7, maximal phosphatidylinositol 3-kinase is bound to Fer within 1 min of insulin receptor activation and is not diminished for at least 20 min. Little if any activity is found bound to Fer preimmune serum.

FIG. 5. Complex formation between Fer and IRS-1 in 3T3-L1 adipocytes. Lysates were prepared from serum-starved 3T3-L1 adipocytes or cells that were stimulated with insulin for 5 min. Lysates were immunoprecipitated with 25 or 50 µl of preimmune serum and anti-FerN serum. Immunoprecipitated proteins were separated on 7.5% SDS-PAGE and transferred to PVDF. Membranes were immunoblotted with either anti-phosphotyrosine antibody (top panel), p85 monoclonal antibody (middle panel), or anti-FerN serum (bottom panel).

FIG. 6. Time course of Fer/IRS-1/p85 complex formation in insulin-stimulated 3T3-L1 adipocytes. Lysates from serum-starved and insulin-stimulated 3T3-L1 adipocytes were immunoprecipitated (IP) with anti-FerN serum. Immunoprecipitated proteins were separated on 7.5% SDS-PAGE and transferred to PVDF. Membranes were immunoblotted with either anti-phosphotyrosine (4G10) antibody (top panel) or anti-p85 monoclonal antibody (bottom panel).

FIG. 7. Insulin-stimulated PI 3-kinase activity associated with anti-Fer immunoprecipitates. Lysates from serum-starved and insulin-stimulated 3T3-L1 adipocytes were immunoprecipitated with anti-Fer or preimmune serum. Immunoprecipitates were assayed for in vitro PI 3-kinase activity as described under “Experimental Procedures.” A, autoradiography of thin layer chromatography plates. B, spots corresponding to $^{32}\text{P}$-labeled phosphoinostitol 3-phosphates were excised and counted in a scintillation counter. Circles represent PI 3-kinase activity associated with Fer immunoprecipitates; triangles represent activity associated with immunoprecipitates with preimmune serum.

DISCUSSION

Here we show that two isoforms of Fer are highly expressed upon 3T3-L1 differentiation. In 3T3-L1 adipocytes, we found a unique splicing variant, iFer, which displays a 65-base pair deletion in the Fer open reading frame. The finding that iFer deletion encodes a protein without a functional kinase indicates that the iFer protein probably has a role in regulating some aspect of Fer function. There are many examples in which alternative splicing of genes produce truncated regulatory forms of proteins. In one case, alternate splicing produces a 26-base pair deletion in the message for the growth hormone receptor that encodes a truncated protein that lacks most of its intracellular domain. This splicing variant produces a soluble circulating form of the growth hormone receptor, which regulates the ability of growth hormone to activate its cellular receptor (19). More recently a truncated splicing variant of protein kinase C δ containing 83-base pair insertion variant was identified. Similar to iFer this splicing variant PKC δIII produces a protein that lacks a functional kinase domain, but retains the regulatory domain (20). The role of this truncated protein in PKC regulation has yet to be determined.

Because iFer retains N-terminal coiled-coil domains, one possible function of iFer could be to regulate Fer oligomerization and activation. It has been hypothesized that like receptor tyrosine kinases, oligomerization activates Fer through the trans-phosphorylation of subunits. In the case of Fes, incubation of wild type full-length Fes with isolated N-terminal domains does in fact suppress Fes autophosphorylation (21). However, in a different study, an N-terminal deletion mutant of Fer, which cannot oligomerize, was found to retain the abil-
ity to autophosphorylate itself. Furthermore, overexpression of the N-terminal region of Fer in COS-1 cells had no effect on Fer activity in in vitro kinase assays (2). Here we show that in COS-1 cells, overexpressed iFer that immunoprecipitates with Fer is tyrosine phosphorylated. This result is consistent with iFer not regulating Fer kinase activity. Nevertheless, it is unclear whether these results reflect an actual biochemical difference between how Fes and Fer are activated or whether they actually reflect a difference because of the experimental design. Another possible function of iFer is to regulate the binding of Fer to other proteins. In initial experiments, we have been able to detect a small amount of iFer associated with both p85 and IRS-1 (data not shown), but this is probably due to an association with full-length Fer in these complexes, as opposed to a direct binding to either IRS-1 or p85. It might be possible that iFer regulates the binding of the N-terminal domain of Fer to the cellular adhesion protein p120<sup>ααα</sup>, which could possibly prevent its tyrosine phosphorylation in response to growth factor stimulation. Further studies are needed to determine exactly what targets this Fer domain might bind in 3T3-L1 adipocytes.

The 94-kDa full-length isoform of Fer is a ubiquitously expressed protein and has been found in almost every cell line studied (6,7). However, there are other cases where Fer expression is increased. Fer has been shown to be absent in pre-B cells but accumulates upon their induction to antibody producing cells (22). Fer expression has also shown to be enhanced in cell extracts from human prostate cancer cell lines (23). The increased expression in differentiated 3T3-L1 cells might indicate that Fer has a role in promoting adipogenesis. Alternatively, this might indicate that Fer has a role in regulating insulin signaling pathways in adipocytes.

In the current work, we show that in 3T3-L1 adipocytes insulin, like PDGF, results in the association of Fer with tyrosine phosphorylated signaling complexes. In 3T3-L1 adipocytes, PDGF results in the binding of Fer to PDGF receptors, whereas insulin activation results in complex formation between Fer and tyrosine phosphorylated IRS molecules. We also show that Fer binds to complexes containing phosphatidylinositol 3-kinase. In the case of PDGF stimulation, it is likely that this complex formation is the result of the binding of both Fer and p85 SH2 domains to the same tyrosine phosphorylated PDGF receptors. However, we cannot rule out the possibility that the SH2 domain of p85 binds directly to tyrosine phosphorylated Fer. Because there is little if any increase of Fer tyrosine phosphorylation in response to insulin, it seem unlikely insulin sensitive complex formation between Fer and p85 is the result of this sort of direct interaction. It is most likely that Fer binds to p85 through tyrosine phosphorylated adaptors such as IRS-1. We cannot rule out the possibility that Fer binds constitutively to IRS-1, because the amount of IRS-1 bound to Fer was below the sensitivity that we are able to detect on immunoblots probed with anti-IRS-1 antibodies (data not shown). It is also possible that Fer binds to p85 through other adaptor proteins that are tyrosine phosphorylated in response to insulin such as IRS-2.

Although it has been hypothesized that cytoplasmic tyrosine kinases such as Fer may have a role in insulin signaling, very little is actually known about their function (24). For example, insulin stimulation results in both the binding of the cytoplasmic tyrosine kinase Fyn to IRS-1 and c-Cbl and its dissociation from Sirtm (25–27). Yet the significance of these associations has yet to be determined. Our data show that Fer might also have a role in insulin signaling. Although Fer is believed to be involved in cross-talk between PDGF receptors and cellular adhesion complexes, there appears to be differences between how Fer responds to PDGF and insulin stimulation. PDGF, unlike insulin, results in the increase of tyrosine phosphorylation of Fer. This suggests that PDGF but not insulin stimulation results in a increase of the catalytic activity of Fer kinase. Thus, although it is possible that Fer may be involved in both insulin and PDGF signaling, Fer might also mediate signaling pathways in 3T3-L1 adipocytes that are unique to PDGF. Further studies to determine the substrates of Fer in this cell type will help unravel these pathways.

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**REFERENCES**

1. Smithgall, T. E., Rogers, J. A., Peters, K. L., Li, J., Briggs, S. D., Lionberger, J. M., Cheng, H., Shibata, A., Scholtz, B., Schreiner, S., and Dunham, N. (1998) *Crit. Rev. Oncog.* 9, 43–62
2. Craig, A. W., Zirngibl, R., and Greer, P. (1999) *J. Biol. Chem.* 274, 19854–19942
3. Kim, L., and Wong, T. W. (1995) *Mol. Cell. Biol.* 15, 4553–4561
4. Aspenstrom, P. (1997) *Curr. Biol.* 7, 479–487
5. Tian, L., Nelson, D. L., and Stewart, D. M. (2000) *J. Biol. Chem.* 275, 7854–7861
6. Feldman, R. A., Tam, J. P., and Hanafusa, H. (1985) *Mol. Cell. Biol.* 5, 1065–1073
7. MacDonald, I., Levy, J., and Pawson, T. (1985) *Mol. Cell. Biol.* 5, 2543–2551
8. Hao, Q. L., Ferris, D. K., White, G., Heisterkamp, N., Groffen, J. (1991) *Mol. Cell. Biol.* 11, 1180–1183
9. Rosato, R., Velmaat, J. M., Groffen, J., and Heisterkamp, N. (1998) *Mol. Cell. Biol.* 18, 5762–5770
10. Kim, L., and Wong, T. W. (1998) *J. Biol. Chem.* 273, 23542–23548
11. Cech, M. P., and Covera, S. (1999) *J. Biol. Chem.* 274, 1865–1868
12. Singh, H., Clerc, R. G., and LeBowitz, J. H. (1989) *BioTechniques* 11, 208–215
13. Cherniack, A. D., Klarlund, J. K., Conway, B. R., and Czech, M. P. (1995) *J. Biol. Chem.* 270, 1485–1488
14. Feldberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* 132, 6–13
15. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
16. Isakov, S. J., Taha, C., Rose, E., Marcusohn, J., Klip, A., and Skolnik, E. Y. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 10247–10251
17. Reed, B. C., Kaufmann, S. H., Mackall, J. C., Student, A. K., and Lane, M. D. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 4876–4880
18. Garcia de Herreros, A., and Birnbaum, M. J. (1989) *J. Biol. Chem.* 264, 19994–19999
19. Dastert, F., Schrier, M. L., Duquesnoy, P., Duriez, B., Goossens, M., and Asemle, S. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 10723–10728
20. Ueyama, T., Ren, Y., Ohmori, S., Sakai, K., Tamaki, N., and Saito, N. (2000) *Biochem. Cell Biol.* Commun. 78, 557–563
21. Read, R. D., Lionberger, J. M., and Smithgall, T. E. (1997) *J. Biol. Chem.* 272, 18498–18503
22. Halachmy, S., Bern, O., Schreiber, L., Carmel, M., Sharabi, Y., Shoham, J., and Nir, U. (1997) *Oncogene* 14, 2871–2880
23. Allard, P., Zoubeidi, A., Nguyen, T. T., Tessier, S., Tanguay, S., Chevrette, M., Aprikian, A., Chevalier, S. (2000) *Mol. Cell. Endocrinol.* 159, 63–77
24. Kazmaki, M., Watson, B. T., Arternemy, N. O., and Pessin, J. E. (2000) *J. Biol. Chem.* 275, 7167–7175
25. Sun, X. J., Pons, S., Asano, T., Myers, M. G., Glasheen, E., and White, M. F. (1994) *J. Biol. Chem.* 271, 10583–10587
26. Ribon, V., and Saltiel, A. R. (1998) *Biochem. J.* 324, 839–845
27. Salvadori, P., Hanash, C. R., Kido, Y., Imai, Y., and Accili, D. (1998) *J. Biol. Chem.* 273, 6989–6997