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Insulin-Stimulated Oocyte Maturation Requires Insulin Receptor Substrate 1 and Interaction with the SH2 Domains of Phosphatidylinositol 3-Kinase

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***Xenopus* oocytes from unprimed frogs possess insulin-like growth factor I (IGF-I) receptors but lack insulin and IGF-I receptor substrate 1 (IRS-1), the endogenous substrate of this kinase, and fail to show downstream responses to hormonal stimulation. Microinjection of recombinant IRS-1 protein enhances insulin-stimulated phosphatidylinositol (PtdIns) 3-kinase activity and restores the germinal vesicle breakdown response. Activation of PtdIns 3-kinase results from formation of a complex between phosphorylated IRS-1 and the p85 subunit of PtdIns 3-kinase. Microinjection of a phosphonopeptide containing a pYMXM motif with high affinity for the *src* homology 2 (SH2) domain of PtdIns 3-kinase p85 inhibits IRS-1 association with and activation of the PtdIns 3-kinase. Formation of the IRS-1–PtdIns 3-kinase complex and insulin-stimulated PtdIns 3-kinase activation are also inhibited by microinjection of a glutathione *S*-transferase fusion protein containing the SH2 domain of p85. This effect occurs in a concentration-dependent fashion and results in a parallel loss of hormone-stimulated oocyte maturation. These inhibitory effects are specific and are not mimicked by glutathione *S*-transferase fusion proteins expressing the SH2 domains of *ras*-GAP or phospholipase Cγ. Moreover, injection of the SH2 domains of p85, *ras*-GAP, and phospholipase Cγ do not interfere with progesterone-induced oocyte maturation. These data demonstrate that phosphorylation of IRS-1 plays an essential role in IGF-I and insulin signaling in oocyte maturation and that this effect occurs through interactions of the phosphorylated YMXM/YXXM motifs of IRS-1 with SH2 domains of PtdIns 3-kinase or some related molecules.**

Insulin, insulin-like growth factor I (IGF-I), and other polypeptide growth factors regulate a wide range of cellular functions through the activation of membrane receptors with tyrosine kinase activity (8, 13, 28, 49, 61, 63). Mutations in the ATP-binding site (9, 16) and autophosphorylation sites (18, 65) in the β-subunit of the insulin receptor inhibit insulin receptor kinase activity and biological responses. Over the past few years, several of the proteins and enzymes involved in linking the insulin receptor to its final effects have been elucidated. Two of the earliest in the pathway are the insulin and IGF-I receptor substrate 1 (IRS-1) (26, 29, 40, 50, 60, 64) and the enzyme phosphatidylinositol (PtdIns) 3-kinase (8, 19, 51).

IRS-1 is a high-molecular-weight cytosolic protein which possesses over 10 potential tyrosine phosphorylation sites (60). Following insulin- and IGF-I-stimulated phosphorylation, IRS-1 rapidly associates with and activates the cytosolic enzyme PtdIns 3-kinase (2, 3, 22, 60). The interaction between PtdIns 3-kinase and IRS-1 is directed through the highly conserved phosphopeptide sequence motifs (YMXM/YXXM) of IRS-1 and the *src* homology 2 (SH2) domain of the 85-kDa subunit of PtdIns 3-kinase (2, 39, 46, 53). SH2 domains are conserved regions that form specific binding sites for phosphotyrosine residues in certain amino acid sequence motifs (reviewed in reference 30). The p85 regulatory subunit of PtdIns 3-kinase contains two SH2 domains and one SH3 domain (20, 42, 55). PtdIns 3-kinase binds to specific tyrosine phosphorylation motifs in IRS-1 and various growth factor receptors via these SH2 domains (reviewed in reference 8). Other SH2 domain-containing proteins, such as $pp60^{v\text{-}src}$, phospholipase Cγ (PLCγ) and the *ras*-associated GTPase-activating protein (*ras*-GAP), bind to the epidermal growth factor, platelet-derived growth factor, and colony-stimulating factor 1 receptors and the polyoma middle T antigen at similar, but not identical, sequence motifs (reviewed in references 8, 21, 25, 30, 36, and 43). Little is known about the function of interactions through these SH2-containing proteins in the insulin signaling pathway.

Xenopus oocytes taken from unprimed frogs possess IGF-I receptors but lack IRS-1 and have little or no response to insulin or IGF-I. Recently, we demonstrated a critical functional role for IRS-1 in insulin- and IGF-I-induced oocyte maturation by microinjecting recombinant IRS-1 protein and reconstituting the germinal vesicle breakdown response (10). To examine the exact molecular interactions required downstream of IRS-1, in the present study, we have used this reconstitution system to evaluate the role of PtdIns 3-kinase and the interaction of IRS-1-containing YMXM/YXXM motifs and the SH2 domain of p85 of PtdIns 3-kinase.

MATERIALS AND METHODS

Oocyte preparation and microinjection. Stage VI oocytes were isolated by mild collagenase treatment of ovaries from

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gravid *Xenopus laevis* frogs (Nasco, Fort Atkinson, Wis.) without previous priming with gonadotropin (6). The oocytes were allowed to recover overnight in modified Barth's media and were then microinjected with 50 nl of either buffer (125 mM NaCl, 25 mM Tris-HCl [pH 7.6]) alone or combinations of peptides and proteins at appropriate concentrations as indicated.

Recombinant rat liver IRS-1 was produced in a baculovirus expression system and purified to >90% homogeneity as previously described (39). Glutathione *S*-transferase (GST) fusion proteins were produced from bacterial expression vectors (pGEX) containing the SH2 domains of p85 (N terminal, designated GST-nSH2^{p85}), *ras*-GAP (N terminal, GST-nSH2$^{ras\text{-}GAP}$), and PLCγ (N terminal, GST-nSH2$^{PLC\gamma}$) as GST fusion constructs (30). Bacterial lysates were purified on a glutathione-Sepharose column (Pharmacia) as previously described (56). The proteins were then dialyzed extensively against a degassed buffer of 88 mM NaCl and 50 mM Tris-HCl, pH 8.0, aliquoted, and stored at −80°C until use.

Phosphonomethylalanine (Pmp), a nonnatural analog of phosphotyrosine, was used to synthesize a nonhydrolyzable phosphonopeptide of the region containing YMXM of the mouse polyomavirus middle T antigen [EEE(PmP) MPMEDLY] (15). This peptide was >90% pure on the basis of high-pressure liquid chromatography (HPLC) analysis in reverse-phase C-18 columns.

PtdIns 3-kinase assay. PtdIns 3-kinase activity was measured by in vitro phosphorylation of PtdIns as previously described (51) with minor modifications. After microinjection with either peptide or protein combinations, oocytes were treated with insulin or IGF-I at the indicated concentrations at 19°C for 5 min (except as otherwise indicated). The incubation medium was removed, and oocytes were extracted with a lysis buffer containing 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4), 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM Na_3VO_4, 10 mM Na PP_i, 10 mM NaF, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine, 10 μg (each) of aprotinin and leupeptin per ml, 10% glycerol, and 1% Nonidet P-40. Undissolved material was removed by centrifugation at 12,000 × *g* for 5 min. PtdIns 3-kinase activity was then assayed in the immunoprecipitates with either anti-IRS-1 or anti-p85 antibodies which were collected on protein A-Sepharose (Pharmacia). These antibodies were produced as previously described (2, 39).

Western blotting. Oocyte lysates or immunoprecipitates of oocyte extracts for Western blot (immunoblot) analysis were prepared as described above for the lipid kinase assay. The association of IRS-1 and the different SH2 domain-GST fusion proteins was assessed by immunoblotting of oocyte extracts collected on the glutathione beads. After incubation of extracts and the beads for 90 min on ice, the complex was washed with phosphate-buffered saline (PBS)–1% Triton X-100 three times and with PBS and 250 mM NaCl twice. The proteins collected on the protein A-Sepharose or glutathione beads were then released by being boiled in the Laemmli sample buffer, resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) under reducing conditions, transferred onto nitrocellulose membranes in Towbin buffer containing 0.02% SDS, and blotted with the antibodies to phosphotyrosine (50), IRS-1 (39), and the p85 subunit of PtdIns 3-kinase (UBI). Specific bands were visualized by ^{125}I-protein A (ICN) and autoradiography. The relative amount of each protein band was determined by laser densitometric analysis with Image Quant Software (Molecular Dynamics).

Oocyte maturation and germinal vesicle breakdown. Stage VI oocytes were isolated and incubated in modified Barth's medium overnight (6). After microinjection with IRS-1 and/or GST-SH2 fusion proteins, oocytes were incubated for 3 h and then transferred to the media containing insulin at 10 μM. After a 20-h incubation, groups of 15 to 20 oocytes were scored for germinal vesicle breakdown (GVBD) by the appearance of a white spot on the animal pole of the oocyte. In some experiments, this was confirmed by the microscopic absence of nuclear membrane after oocytes were fixed in 5% trichloroacetic acid for 30 min.

MAP kinase activity assay. Groups of oocytes were lysed in a buffer containing 50 mM β-glycerophosphate (pH 7.4), 5 mM $MgCl_2$, 5 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1 mM benzamidine, 0.5 mM Na_3VO_4, 0.1 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, and 1 mM dithiothreitol. Crude cell extracts were aliquoted and stored at −80°C after centrifugation at 13,000 × *g* for 10 min at 4°C. Kinase reaction was performed in the cell lysate (equivalent to 1.5 oocytes per tube) by incubation with [γ-^{32}P]ATP and substrates (0.5 mM of myelin basic protein peptide [UBI]) in the presence of 10 mM $MgCl_2$ and 2 μM protein kinase A inhibitor (Sigma). Reactions were terminated after 15 min at 30°C by adding a 1/2 volume of 2 M HCl. Samples (25 μl) were spotted onto p81 phosphocellulose paper disks (GIBCO) and washed twice in 1% acetic acid and twice in H_2O. The disks were then dried and counted for radioactivity (Cerenkov method). Nonspecific ^{32}P incorporation was determined in identical assays lacking cell lysates. Each measurement was done in duplicate in at least two experiments.

RESULTS

Microinjection of IRS-1 enhances insulin-stimulated PtdIns 3-kinase activation in *Xenopus* oocytes. *Xenopus* oocytes prepared from frogs which have not received gonadotropin treatment possess endogenous IGF-I receptors but have little or no detectable IRS-1 (10, 24, 27, 38). In these oocytes, insulin and IGF-I stimulated only a small increase in PtdIns 3-kinase activity as assayed in immunoprecipitates with either anti-IRS-1 (Fig. 1) or anti-p85 (Fig. 2) antibodies. This activation was maximal after 5 min of insulin stimulation, a relatively slow time course compared with that in mammalian cells (data not shown). Following injection of baculovirus-produced IRS-1 protein (12.5 ng per oocyte), insulin stimulated a fourfold-greater increase in both IRS-1-associated and total PtdIns 3-kinase activities (Fig. 1B and 2). Both insulin and IGF-I stimulated the association of PtdIns 3-kinase with IRS-1 in a concentration-dependent manner, with IGF-I being 100-fold-more potent than insulin, consistent with stimulation via an IGF-I type receptor (Fig. 1B).

The 85-kDa subunit of the endogenous PtdIns 3-kinase in *Xenopus* oocytes could be detected by immunoblotting of immunoprecipitates prepared with 50 to 100 oocytes by using antibodies raised to the mammalian enzyme (Fig. 3, lane j). Association of IRS-1 and p85 of PtdIns 3-kinase, as assessed by coprecipitation of the two proteins with anti-IRS-1, followed a pattern consistent with the enzymatic activity data. In the absence of injected IRS-1, no p85 was observed in anti-IRS-1 precipitates in either the absence or presence of insulin stimulation (Fig. 3, lanes a and b). Following IRS-1 injection and insulin stimulation, a faint p85 band could be detected in anti-IRS-1 precipitates and repre-

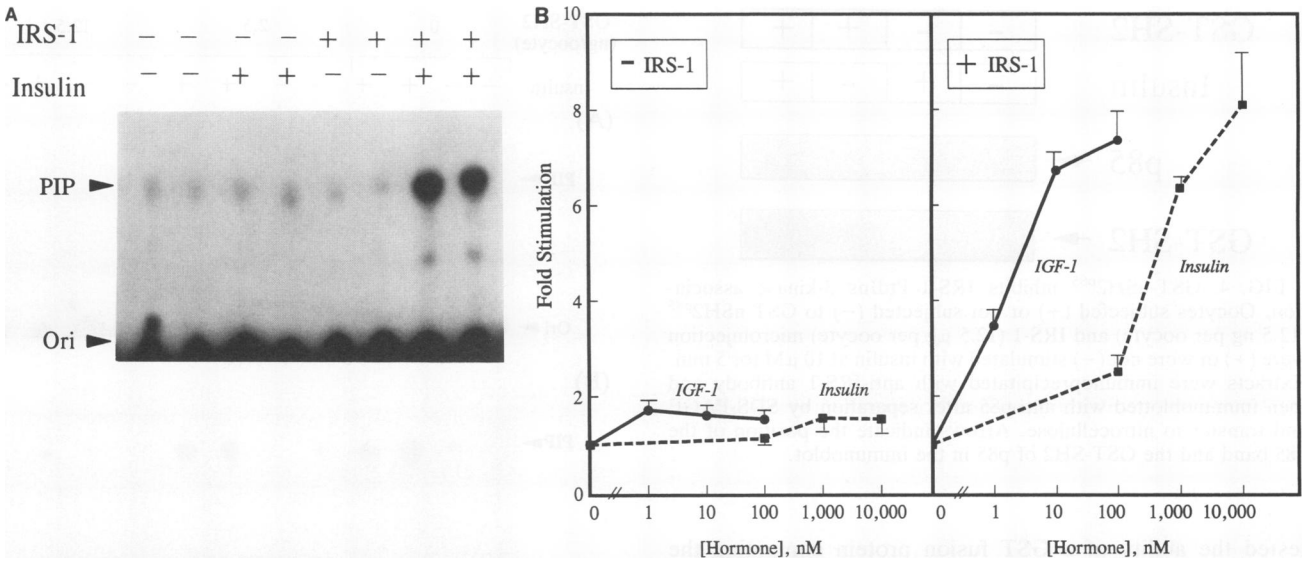

FIG. 1. Effects of IRS-1 on insulin- and IGF-I-stimulated IRS-1-associated PtdIns 3-kinase in *Xenopus* oocytes. Oocytes were or were not microinjected with IRS-1 (12.5 ng per oocyte). Groups of 15 oocytes were lysed after incubation of various concentrations of insulin (closed squares [B]) or IGF-I (closed circles [B]) for 5 min. PtdIns 3-kinase activity was then assayed on the immunoprecipitates of anti-IRS-1 antibody. (A) Thin-layer chromatographs of the extracted lipids from the oocytes treated (+) or not treated (−) with 10 μM insulin for 5 min. The lipids were extracted and separated by thin-layer chromatography in $CHCl_3$-CH_3OH-H_2O-NH_4OH (60:47:11.3:2). Radioactivity of the ^{32}P incorporation into PtdIns(3) phosphate (PIP) was visualized by autoradiography for 3 h. (B) Data were quantified by Cerenkov counting. Data represent the means and standard errors of the mean (bar) of three to five separate experiments, each done in duplicate.

sented about 10% the total PtdIns 3-kinase in the oocyte (Fig. 3, compare lanes d and h). When normalized for the amount of the p85 protein brought down by these antibodies, the specific activity of IRS-1-associated PtdIns 3-kinase after IRS-1 microinjection and insulin stimulation was significantly higher than that in the total pool of PtdIns 3-kinase (17-fold increase in IRS-1-associated PtdIns 3-kinase specific activity compared with a 2.7-fold increase in p85-associated PtdIns 3-kinase specific activity in anti-p85 immunoprecipitates). There was no evidence of tyrosine phosphorylation of p85 on the basis of immunoprecipitation by antiphosphotyrosine antibodies followed by blotting with anti-p85 antibody (Fig. 3, lane i). These findings are consistent with activation of PtdIns 3-kinase by binding to the phosphorylated IRS-1 following insulin stimulation (2, 22, 53).

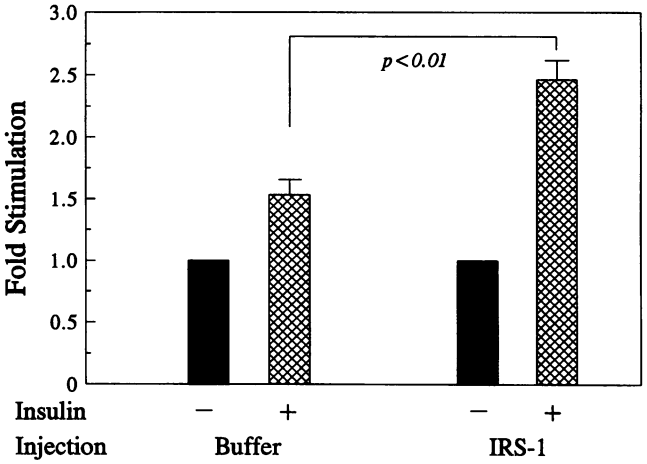

FIG. 2. Effects of IRS-1 on insulin-stimulated p85-associated PtdIns 3-kinase activity in *Xenopus* oocytes. Oocytes microinjected with buffer or IRS-1 were (+) or were not (−) stimulated with insulin at 10 μM. PtdIns 3-kinase activity in the immunoprecipitates was measured by using anti-p85 as described in the legend to Fig. 1. Data were quantitated by Cerenkov counting and present the means plus standard errors of the means of three separate experiments, each in duplicate. The statistical significance was calculated by using the Student *t* test.

An SH2 domain of p85 is important for PtdIns 3-kinase activation and oocyte maturation induced by insulin. To address the functional role of the SH2 domains of the p85 subunit of PtdIns 3-kinase in the interaction with IRS-1, we

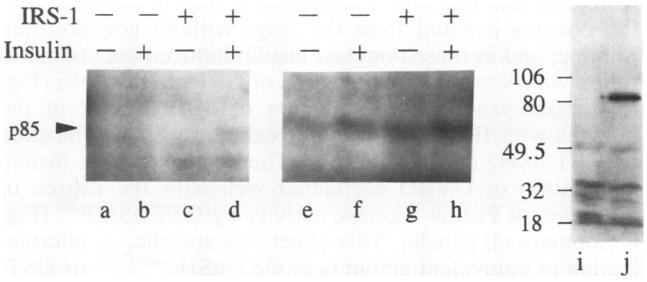

FIG. 3. Association of IRS-1 and p85 of PtdIns 3-kinase upon insulin stimulation. Oocytes microinjected (+) or not injected (−) with IRS-1 were or were not stimulated with insulin at 10 μM for 5 min. The extracts were then immunoprecipitated with either anti-IRS-1 (lanes a to d), anti-p85 (lanes e to h and j), or antiphosphotyrosine (lane i) antibodies. Washed immunoprecipitates were boiled in Laemmli sample buffer and separated by SDS-PAGE. After transfer to nitrocellulose, the filters were immunoblotted with anti-p85 antibody (UBI). The results shown in lanes i and j were from experiments done with 100 oocytes per point. Molecular mass markers (in kilodaltons) are on the left. The experiments shown in lanes a to h were performed with 50 oocytes per lane. The position of p85 is indicated by an arrow.

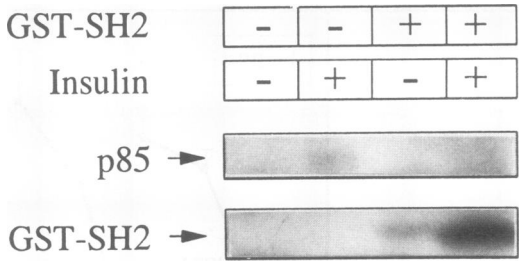

FIG. 4. GST-nSH2^{p85} inhibits IRS-1–PtdIns 3-kinase association. Oocytes subjected (+) or not subjected (−) to GST-nSH2^{p85} (12.5 ng per oocyte) and IRS-1 (12.5 μg per oocyte) microinjection were (+) or were not (−) stimulated with insulin at 10 μM for 5 min. Extracts were immunoprecipitated with anti-IRS-1 antibody and then immunoblotted with anti-p85 after separation by SDS-PAGE and transfer to nitrocellulose. Arrows indicate the position of the p85 band and the GST-SH2 of p85 in the immunoblot.

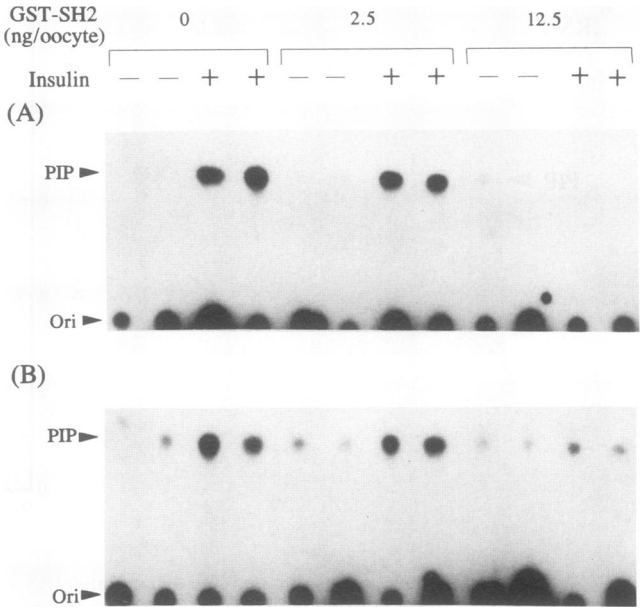

FIG. 5. GST-nSH2^{p85} inhibits insulin-stimulated PtdIns 3-kinase activities. Oocytes were coinjected with different amounts of GST-nSH2^{p85} and 12.5 ng of IRS-1 per oocyte. Insulin-stimulated PtdIns 3-kinase activity was assayed in the immunoprecipitates of anti-IRS-1 (A) and anti-p85 (B) antibodies as described in the legend to Fig. 1. The ^{32}P incorporated into PtdIns(3) phosphate (PIP) was visualized by autoradiography.

tested the ability of a GST fusion protein containing the N-terminal SH2 domain of the p85 (GST-nSH2^{p85}) to inhibit the association between IRS-1 and PtdIns 3-kinase in the oocytes. As noted above, following injection of IRS-1 into the oocytes and insulin stimulation, immunoprecipitation with anti-IRS-1 antibody resulted in coprecipitation of the p85 subunit of PtdIns 3-kinase with IRS-1 molecules, suggesting that p85 and IRS-1 form a stable complex during insulin stimulation (Fig. 4, second lane from left). Formation of this complex was blocked by comicroinjection of GST-nSH2^{p85} (Fig. 4, far right lane). Furthermore, the presence of a large amount of GST-SH2 protein in the anti-IRS-1 immunoprecipitates indicates that the GST-SH2 protein bound to the phosphorylated YMXM sites in IRS-1, preventing the binding of the PtdIns 3-kinase. As a result, both the binding of PtdIns 3-kinase to IRS-1 and its activation upon insulin stimulation were inhibited by GST-nSH2^{p85} when assayed in either the anti-IRS-1 (Fig. 5A) or the anti-p85 (Fig. 5B) immunoprecipitates. This effect occurred in a concentration-dependent fashion, and blockade was almost complete when the GST-SH2 protein was injected in a three- to fourfold molar excess over the IRS-1.

As previously observed (10), IRS-1 microinjection enhances insulin and IGF-I induction of oocyte maturation in the oocytes isolated from the frogs without gonadotropin priming, and in these oocytes, insulin-induced oocyte maturation was blocked by coinjection of GST-nSH2 of p85 (Fig. 6, bottom panel). The activation of MAP kinase in the oocytes with IRS-1 and insulin treatment was also blocked by GST-nSH2 of p85 (Table 1). The decrease in the insulin stimulation of GVBD correlated well with the degree of inhibition of PtdIns 3-kinase activity by GST-nSH2^{p85} (Fig. 6, compare all panels). This effect was specific, as microinjection of equivalent amounts of GST-nSH2$^{ras\text{-}GAP}$ or GST-nSH2$^{PLC\gamma}$ did not inhibit the insulin-stimulated PtdIns 3-kinase activation and oocyte maturation responses (Fig. 7). Furthermore, progesterone-induced oocyte maturation was not affected by microinjection of any of the different SH2 domain proteins, including that of p85 of PtdIns 3-kinase (data not shown).

Effect of GST-nSH2^{p85} is not due to decreasing stability and tyrosine phosphorylation of the injected IRS-1. Chronic exposure of mammalian cells to insulin results in a down-regulation of IRS-1 (47, 52). In *Xenopus* oocytes, insulin treatment also appears to increase the degradation of injected IRS-1 as determined by immunoblotting of cell extracts (Fig. 8A, lanes e and f). To rule out the possibility that inhibition of oocyte maturation by GST-nSH2^{p85} was due to decreased stability and/or decreased tyrosine phosphorylation of IRS-1, we studied the effect of GST-nSH2^{p85} on IRS-1 stability and phosphorylation. Microinjection of increasing amounts of GST-nSH2^{p85} appears to actually enhance both the stability and tyrosine phosphorylation of injected IRS-1 after insulin stimulation (Fig. 8). This suggests that the binding of GST-nSH2^{p85} protein protects phosphorylated IRS-1 from the actions of some proteases and/or phosphotyrosine phosphatases present in the oocyte.

IRS-1 binds p85 of PtdIns 3-kinase through phosphorylated YMXM/YXXM motifs. Previous work has suggested that IRS-1 interacts with the SH2 domains of PtdIns 3-kinase via the repetitive tyrosine-phosphorylated YMXM and YXXM motifs (2, 39, 46, 60, 66). To test this possibility directly, phosphorylated and unphosphorylated peptides containing YMXM motifs from IRS-1 or related proteins were microinjected into *Xenopus* oocytes. However, these peptides were rapidly degraded by endogenous proteases and/or phosphatases and appeared to have no effect on IRS-1 association of PtdIns 3-kinase (data not shown). Therefore, we prepared a phosphonopeptide containing a YMXM motif known to have high affinity for the p85 of PtdIns 3-kinase by using the nonhydrolyzable phosphotyrosine analog Pmp. The half-life of this peptide after microinjection into the oocytes was about 1 h as shown by the HPLC analysis of the iodinated peptide (data not shown). Furthermore, the presence of the phosphonopeptide blocked insulin-stimulated PtdIns 3-kinase activation for 1 and 2 h after microinjection (Fig. 9). These data support the notion that the phosphorylated YMXM/YXXM motifs in IRS-1 are the major site of binding to the p85 of PtdIns 3-kinase.

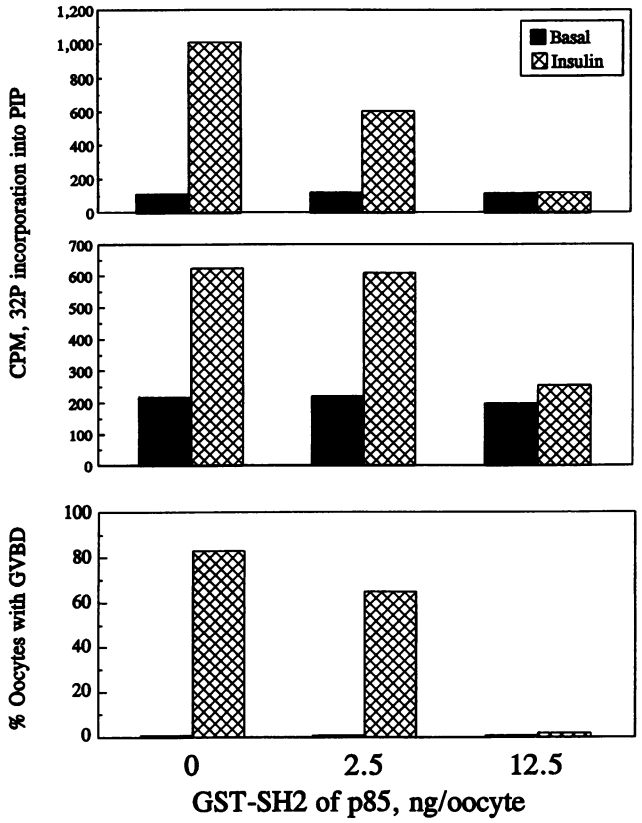

FIG. 6. GST-nSH2^{p85} inhibits insulin-stimulated PtdIns 3-kinase activity and oocyte maturation. Oocytes were coinjected with different amounts of GST-nSH2^{p85} and 12.5 ng of IRS-1 per oocyte. IRS-1-associated and total PtdIns 3-kinase activities were measured and quantified by Cerenkov counting (the upper and middle panels, respectively) as described in the legend to Fig. 5. The maturation response of the oocyte (bottom panel) was scored in a group of 15 to 20 oocytes by the presence of a white spot on the animal hemisphere (GVBD). Data represent the means of two separate experiments. Basal and Insulin, stimulation with 0 and 10 μM insulin, respectively. PIP, PtdIns phosphate.

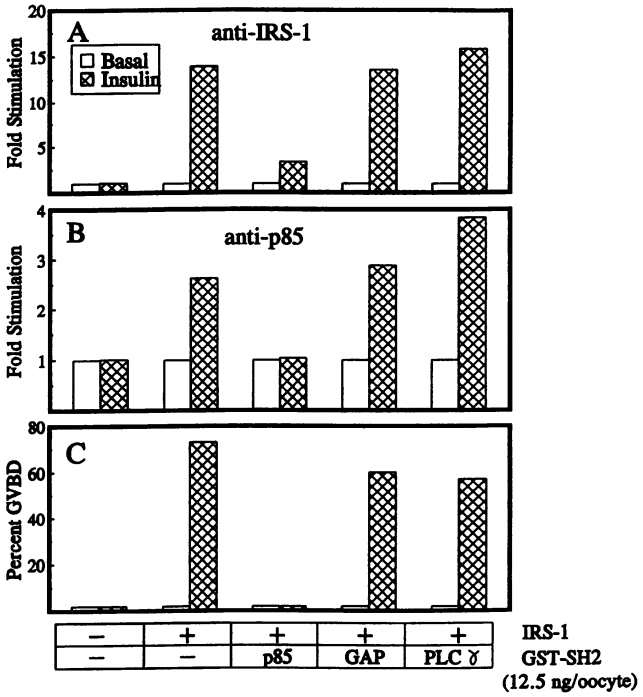

FIG. 7. Effects of GST-SH2 domains of p85, *ras*-GAP, and PLCγ on insulin-stimulated PtdIns 3-kinase activation and oocyte maturation. Oocytes were microinjected with 12.5 ng of IRS-1 and 12.5 ng of GST-SH2 of the different proteins. PtdIns 3-kinase activities were measured after insulin stimulation for 5 min in anti-IRS-1 (A) and anti-p85 (B) immunoprecipitates as described above. (C) Oocyte maturation response was assayed 16 to 20 h after transferring to insulin (10 μM)-containing modified Barth's media. Data represent the means of two separate experiments. Basal and Insulin, stimulation with 0 and 10 μM insulin, respectively.

DISCUSSION

Xenopus oocytes provide a unique system for reconstitution of the insulin and IGF-I action cascade. Previous studies have shown that *Xenopus* oocytes possess many of the serine/threonine kinases involved in the insulin action cascade (7, 33, 35, 44, 58) and possess IGF-I receptors (24, 27, 34); however, presence of the novel PtdIns 3-kinase has not been reported. In this study, we demonstrated that PtdIns 3-kinase exists in *Xenopus* oocytes and can be activated by insulin and IGF-I via the IRS-1 protein. Furthermore, IRS-1 associates with PtdIns 3-kinase through an interaction between YMXM/YXXM sequence motifs and SH2 domains, and inhibition of this association selectively blocks not only insulin-stimulated PtdIns 3-kinase activation but also oocyte maturation.

Over the past decade, considerable evidence has indicated that insulin and IGF-I exert their functions through the binding and activation of their receptor tyrosine kinases. By using antiphosphotyrosine antibodies, several proteins have been implicated as potential substrates for the kinases, including pp185/IRS-1 (26, 29, 64), pp120 (37, 45), pp46 (25), and pp15 (5). Recently, we purified and cloned IRS-1 from rat liver and human muscle at the cDNA level and from mice, rats, and humans at the genomic level (1, 50, 60). On the basis of the deduced sequence, IRS-1 is a unique signaling molecule with multiple tyrosine phosphorylation sites in YMXM or YXXM motifs which act as docking sites for SH2 domain proteins, particularly the SH2 of the 85-kDa subunit of the PtdIns 3-kinase. All of the potential tyrosine phosphorylation sites and >90% of the remainder of the protein sequence are conserved among the three species studied (1, 41). Expression of IRS-1 in CHO cells enhances insulin stimulation of thymidine incorporation, suggesting a role for IRS-1 in insulin-stimulated mitogenic responses (59).

Xenopus oocytes possess IGF-I receptors and, under some circumstances, respond to insulin or IGF-I with a modest stimulation of the maturation response (4, 11, 17, 23,

TABLE 1. Changes in MAP kinase activity during oocyte maturation: effects of IRS-1 and GST-SH2 of p85[a]

Injection	MAP kinase (fold stimulation)	
	Without insulin	With insulin
None	1	1.74 ± 0.38
IRS-1	1	3.86 ± 1.18
IRS-1 + SH2^{p85}	1	1.21 ± 0.04

[a] Data are presented as means ± standard errors (n = 4).

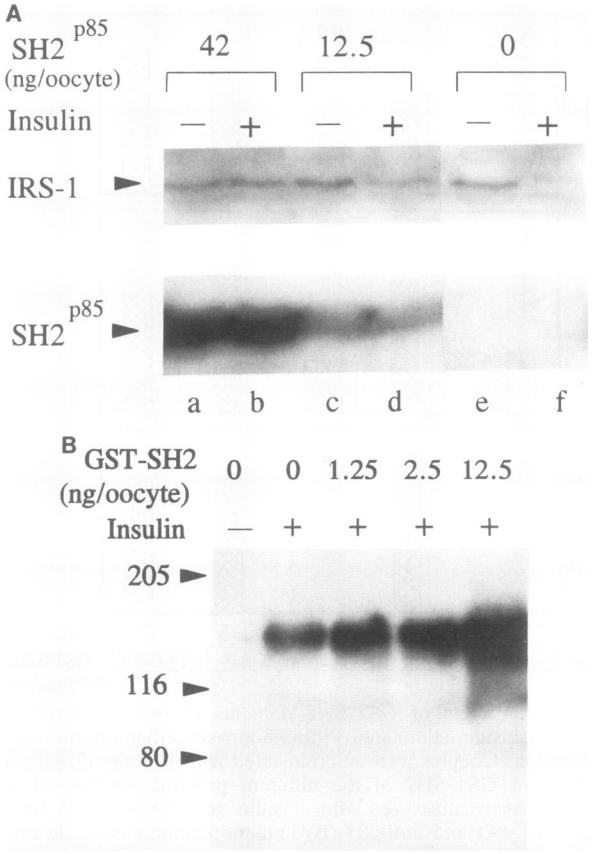

FIG. 8. Effects of GST-nSH2^{p85} on the stability and tyrosine phosphorylation of IRS-1 after insulin stimulation. Oocytes were microinjected with 12.5 ng of IRS-1 and various amounts of GST-nSH2^{p85}. Extracts of oocytes after insulin stimulation overnight were then separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with both anti-IRS-1 and anti-p85 antibodies (A) and antiphosphotyrosine antibodies (B). Specific bands corresponding IRS-1 and GST-nSH2^{p85} were visualized by ^{125}I-protein A and autoradiography. (B) Molecular mass markers (in kilodaltons) are on the left.

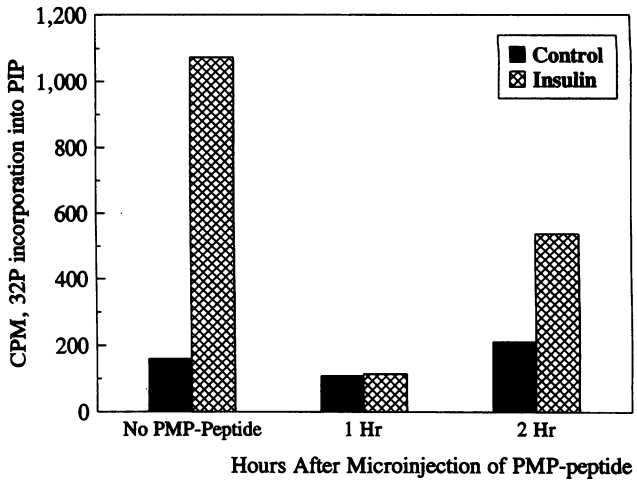

FIG. 9. Nonhydrolyzable Pmp-phosphonopeptide of middle T antigen inhibits insulin-stimulated association of IRS-1 and PtdIns 3-kinase. Oocytes were coinjected with IRS-1 (12.5 ng per oocyte) and the Pmp-phosphonopeptide (0.5 pmol per oocyte) of middle T antigens as described in Materials and Methods. One to 2 h after microinjection, oocytes were lysed and IRS-1-associated PtdIns 3-kinase activity was measured. Data are the means of two experiments.

24, 31, 32, 34, 62). Insulin and IGF-I signalings in these oocytes involve tyrosine phosphorylation and can be blocked by microinjection of antiphosphotyrosine antibody or phosphotyrosine phosphatases (12, 23). Several phosphotyrosyl proteins have been implicated in these signaling events, including the IGF-I receptor, the MPF component p34^{cdc2}, MAP kinases, and the *Xenopus* homolog of pp60^{src} (12, 33). Although a 160-kDa phosphotyrosine protein has been reported in the microsomal fraction of the *Xenopus* oocyte (12), in oocytes isolated from the frogs without gonadotropin priming, we find little or no IRS-1 upon immunoblotting with any of three anti-IRS-1 antibodies (anti-C terminal, anti-N terminal, or anti-pep80) or with antiphosphotyrosine antibodies (10). Likewise, in the present study, we find little or no IRS-1-associated PtdIns 3-kinase activation in unprimed oocytes. Of course, we cannot rule out the possibility that our antibodies do not cross-react with frog IRS-1 or that the protein is of low abundance and undetectable by these means.

Following microinjection of recombinant IRS-1, there is a rapid insulin and IGF-I stimulation of PtdIns 3-kinase which parallels receptor autophosphorylation and tyrosine phosphorylation of the injected IRS-1 protein. The oocyte maturation response induced by insulin also correlated well with PtdIns 3-kinase activation following IRS-1 injection.

The interaction between IRS-1 and the PtdIns 3-kinase is mediated via the SH2 domains of the p85 of PtdIns 3-kinase. Thus, insulin-stimulated PtdIns 3-kinase activity and the GVBD response are blocked by GST-containing the N-terminal SH2 domain of p85 but not by the GST-SH2 of *ras*-GAP and PLCγ. Microinjected GST-nSH2^{p85} also blocks the association of phosphorylated IRS-1 with p85. This inhibitory action of the GST fusion protein is not due to an effect on the stability or tyrosine phosphorylation of IRS-1. Indeed, the stability and tyrosine phosphorylation of the IRS-1 are actually enhanced following injection of the GST-SH2^{p85}. Furthermore, IRS-1 and/or GST-SH2 microinjection had no effect on the ability of progesterone to stimulate oocyte maturation, and progesterone had no effect on PtdIns 3-kinase activity.

The interaction of IRS-1 and p85 subunit of PtdIns 3-kinase is also mediated through the YMXM/YXXM sequence motifs of the IRS-1 protein. Phosphopeptides containing such motifs bind to the p85 and lead to a conformational change and activation of PtdIns 3-kinase in vitro (2, 39, 46, 53). The data of the present study show that this interaction also occurs inside the oocytes. Peptides containing YMXM sequences with a nonhydrolyzable derivative of phosphotyrosine inhibit the IRS-1 association with and activation of the PtdIns 3-kinase which follows insulin stimulation. However, the effect of the injected peptide is only transient in the oocyte because of peptide degradation. Thus, the transmission of specific insulin signals depends on interaction of IRS-1 with SH2-containing signaling molecules and is blocked by inhibiting the binding of this class of molecules to IRS-1.

Both progesterone and insulin can induce oocyte maturation through separate, but converging, signaling pathways (33, 57). One central element controlling G_2-to-M-phase transition in the cell cycle is the maturation-promoting factor

consisting of $p34^{cdc2}$ and cyclin B_2. Exactly how insulin IRS-1 and PtdIns 3-kinase signals are transmitted to these events has not been elucidated. On the basis of other studies, it seems likely that $p21^{ras}$, Raf-1, MAP kinase, MAP kinase kinase, and $pp90^{rsk}$ are evolved (reference 48 and references therein). Although there are some conflicting results in establishing a relationship between the activation of Ras and PtdIns 3-kinase (14, 54), recent data from platelet-derived growth factor receptor studies suggested that PtdIns 3-kinase lies upstream of Ras activation (21). If this is also true for the insulin receptor–IRS-1 system, it is possible that inhibition of PtdIns 3-kinase by microinjection of GST-$nSH2^{p85}$ blocks oocyte maturation by interfering with the activation of *ras*-dependent kinases. Whatever the exact mechanism, the present study demonstrates the critical role of IRS-1 and its association and activation of PtdIns 3-kinase via YMXM/YXXM motifs and SH2 domains in the insulin and IGF-I response.

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REFERENCES

1. **Araki, E., X.-J. Sun, B. L. Haag, L.-M. Chuang, Y. Zhang, T. L. Yang-Feng, M. F. White, and C. R. Kahn.** 1993. Human skeletal muscle insulin receptor substrate 1 (IRS-1): characterization of the cDNA, gene and chromosomal localization. Diabetes **42:** 1041–1054.
2. **Backer, J. M., M. G. Myers, S. E. Shoelson, D. J. Chin, X. J. Sun, M. Miralpeix, P. Hu, B. Margolis, Y. Skolnik, J. Schlessinger, and M. F. White.** 1992. Phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. EMBO J. **11:**3469–3479.
3. **Backer, J. M., G. C. Schroeder, C. R. Kahn, M. G. Myers, P. A. Wilden, D. A. Cahill, and M. F. White.** 1992. Insulin stimulation of phosphatidylinositol 3-kinase activity maps to insulin receptor regions required for endogenous substrate phosphorylation. J. Biol. Chem. **267:**1367–1374.
4. **Barrett, C. B., R. M. Schroetke, F. A. Van der Hoorn, S. K. Nordeen, and J. L. Maller.** 1990. Ha-$ras^{Val\text{-}12,Thr\text{-}59}$ activates S6 kinase and $p34^{cdc2}$ kinase in *Xenopus* oocytes: evidence for c-mos^{xe}-dependent and -independent pathways. Mol. Cell. Biol. **10:**310–315.
5. **Bernier, M., D. M. Laird, and M. D. Lane.** 1987. Insulin-activated tyrosine phosphorylation of a 15-kilodalton protein in intact 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. USA **84:**1844–1848.
6. **Birnbaum, M. J.** 1989. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. Cell **57:**305–315.
7. **Campa, M. J., J. F. Glickman, K. Yamamoto, and K.-J. Chang.** 1992. The antibiotic azatyrosine suppresses progesterone or [Val12]p21 Ha-ras/insulin-like growth factor 1-induced germinal vesicle breakdown and tyrosine phosphorylation of *Xenopus* mitogen-activated protein kinase in oocytes. Proc. Natl. Acad. Sci. USA **89:**7654–7658.
8. **Cantley, L. C., K. R. Auger, C. Carpenter, B. Duckworth, R. Kapeller, and S. Soltoff.** 1991. Oncogenes and signal transduction. Cell **64:**281–302.
9. **Chou, C. K., T. J. Dull, E. S. Russell, R. Gherzi, D. Lebwohl, A. Ullrich, and O. M. Rosen.** 1987. Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. J. Biol. Chem. **262:**1842–1847.
10. **Chuang, L. M., M. G. Myers, Jr., G. A. Seidner, M. J. Birnbaum, M. F. White, and C. R. Kahn.** 1993. Insulin receptor substrate-1 (IRS-1) mediates insulin and IGF-I stimulated maturation of *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA **90:**5172–5175.
11. **Cicirelli, M. F., S. L. Pelech, and E. G. Krebs.** 1988. Insulin and progesterone activate a common synthetic ribosomal protein S6 peptide kinase in *Xenopus* oocytes. FEBS Lett. **241:**195–201.
12. **Cicirelli, M. F., N. K. Tonks, C. D. Diltz, J. E. Weiel, E. H. Fischer, and E. G. Krebs.** 1990. Microinjection of a protein-tyrosine phosphatase inhibits insulin action in Xenopus oocytes. Proc. Natl. Acad. Sci. USA **87:**5514–5518.
13. **Czech, M. P.** 1989. Signal transmission by the insulin-like growth factors. Cell **59:**235–238.
14. **de Vries-Smits, A. M. M., B. M. T. Burgering, S. J. Leevers, C. J. Marshall, and J. L. Bos.** 1992. Involvement of $p21^{ras}$ in activation of extracellular signal-regulated kinase 2. Nature (London) **357:**602–604.
15. **Domchek, S. M., K. R. Auger, S. Chatterjee, T. R. Burke, Jr., and S. E. Shoelson.** 1992. Inhibition of SH2 domain/phosphoprotein association by a nonhydrolyzable phosphonopeptide. Biochemistry **31:**9865–9870.
16. **Ebina, Y., E. Araki, M. Taira, F. Shimada, M. Mori, C. S. Craik, K. Siddle, S. B. Pierce, R. A. Roth, and W. J. Rutter.** 1987. Replacement of lysine residue 1030 in the putative ATP-binding region of the insulin receptor abolishes insulin- and antibody-stimulated glucose uptake and receptor kinase activity. Proc. Natl. Acad. Sci. USA **84:**704–708.
17. **El-Etr, M., S. Schorderet-Slatkine, and E. E. Baulieu.** 1979. Meiotic maturation in *Xenopus laevis* oocytes initiated by insulin. Science **205:**1397–1399.
18. **Ellis, L., E. Clauser, D. O. Morgan, M. Edery, R. A. Roth, and W. J. Rutter.** 1986. Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. Cell **45:**721–732.
19. **Endermann, G., K. Yonezawa, and R. A. Roth.** 1990. Phosphatidylinositol kinase or an associated protein is a substrate for the insulin receptor kinase. J. Biol. Chem. **265:**396–400.
20. **Escobedo, J. A., S. Navankasattusas, W. M. Kavanaugh, D. Milfay, V. A. Fried, and L. T. Williams.** 1991. cDNA cloning of a novel 85 kD protein that has SH2 domains and regulates binding of PI 3-kinase to the PDGF β-receptor. Cell **65:**75–82.
21. **Fantl, W. J., J. A. Escobedo, G. A. Martin, C. W. Turck, M. DeRosario, F. McCormic, and L. T. Williams.** 1992. Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. Cell **69:** 413–423.
22. **Folli, F., M. J. A. Saad, J. M. Backer, and C. R. Kahn.** 1992. Insulin stimulation of phosphatidylinositol 3-kinase activity and association with IRS-1 in liver and muscle of the intact rat. J. Biol. Chem. **267:**22171–22177.
23. **Hainaut, P., S. Giorgetti, A. Kowalski, R. Ballotti, and E. Van Obberghen.** 1991. Antibodies to phosphotyrosine injected in Xenopus laevis oocytes modulate maturation induced by insulin/IGF-I. Exp. Cell Res. **195:**129–136.
24. **Hainaut, P., A. Kowalski, S. Giorgetti, V. Baron, and E. Van Obberghen.** 1991. Insulin and insulin-like-growth-factor-I (IGF-I) receptors in Xenopus laevis oocytes. Comparison with insulin receptors from liver and muscle. Biochem. J. **273:**673–678.
25. **Heldin, C. H.** 1991. SH2 domains: elements that control protein interactions during signal transduction. Trends Biochem. Sci. **16:**450–452.
26. **Izumi, T., M. F. White, T. Kadowaki, F. Takaku, Y. Akanuma, and M. Kasuga.** 1988. Insulin-like growth factor I rapidly stimulates tyrosine phosphorylation of a Mr 185,000 protein in intact cells. J. Biol. Chem. **262:**1282–1287.
27. **Janicot, M., J. R. Flores-Riveros, and M. D. Lane.** 1991. The insulin-like growth factor 1 (IGF-I) receptor is responsible for mediating the effects of insulin, IGF-I, and IGF-2 in Xenopus laevis oocytes. J. Biol. Chem. **266:**9382–9391.

28. **Kasuga, M., F. Karlsson, and C. R. Kahn.** 1982. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. Science **215:**185–189.
29. **Keller, S. R., K. Kitagawa, R. Aebersold, G. E. Lienhard, and C. W. Garner.** 1991. Isolation and characterization of the 160,000-Da phosphotyrosyl protein, a putative participant in insulin signaling. J. Biol. Chem. **266:**12817–12820.
30. **Koch, C. A., D. Anderson, M. F. Moran, C. Ellis, and T. Pawson.** 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. Science **252:**668–674.
31. **Korn, L. J., C. W. Siebel, F. McCormic, and R. A. Roth.** 1987. Ras p21 as a potential mediator of insulin action in *Xenopus* oocytes. Science **236:**840–843.
32. **LeGoascogne, C., S. Hirai, and E. E. Baulieu.** 1984. Induction of germinal vesicle breakdown in *Xenopus laevis* oocytes: synergistic action of progesterone and insulin. J. Endocrinol. **101:**7–12.
33. **Maller, J. L.** 1990. Xenopus oocytes and the biochemistry of cell division. Biochemistry **29:**3157–3166.
34. **Maller, J. L., and J. W. Koontz.** 1981. A study of the induction of cell division in amphibian oocytes by insulin. Dev. Biol. **84:**309–316.
35. **Maller, J. L., L. J. Pike, G. R. Freidenberg, R. Cordera, B. J. Stith, J. M. Olefsky, and E. G. Krebs.** 1986. Increased phosphorylation of ribosomal protein S6 following microinjection of insulin receptor-kinase into *Xenopus* oocytes. Nature (London) **320:**459–461.
36. **Margolis, B.** 1992. Proteins with SH2 SH3 domains: transducers in the tyrosine kinase signaling pathway. Cell Growth Diff. **3:**73–80.
37. **Margolis, R. N., M. J. Schell, S. I. Taylor, and A. L. Hubbard.** 1990. Hepatocyte plasma membrane ecto-ATPase (pp120/HA4) is a substrate for tyrosine kinase activity of the insulin receptor. Biochem. Biophys. Res. Commun. **166:**562–566.
38. **Morgan, D. O., L. Ho, L. J. Korn, and R. A. Roth.** 1986. Insulin action is blocked by a monoclonal antibody that inhibits the insulin receptor kinase. Proc. Natl. Acad. Sci. USA **83:**328–332.
39. **Myers, M. G., Jr., J. M. Backer, X. J. Sun, S. Shoelson, P. Hu, J. Schlessinger, M. Yoakim, B. Schaffhausen, and M. F. White.** 1992. IRS-1 activates phosphatidylinositol 3-kinase by associating with src homology 2 domain of p85. Proc. Natl. Acad. Sci. USA **89:**10350–10354.
40. **Myers, M. G., Jr., X. J. Sun, B. Cheatham, B. R. Jachna, E. M. Glasheen, J. M. Backer, and M. F. White.** 1993. IRS-1 is a common element in insulin and IGF-I signalling to the phosphatidylinositol 3′-kinase. Endocrinology **132:**1421–1430.
41. **Nishiyama, M., and J. R. Wands.** 1992. Cloning and increased expression of an insulin receptor substrate-1-like gene in human hepatocellular carcinoma. Biochem. Biophys. Res. Commun. **183:**280–285.
42. **Otsu, M., I. Hiles, I. Gout, M. J. Fry, F. Ruiz-Larrea, G. Panayotou, A. Thompson, R. Dhand, J. Hsuan, N. Totty, A. D. Smith, S. J. Mogan, S. A. Courtneidge, P. J. Parker, and M. D. Waterfield.** 1991. Characterization of two 85 kD proteins that associate with receptor tyrosine kinase, middle-T/pp60$^{c\text{-}src}$ complexes, and PI 3-kinase. Cell **65:**91–104.
43. **Pawson, T.** 1992. SH2 and SH3 domains. Curr. Opin. Struct. Biol. **2:**432–437.
44. **Pelech, S. L., and J. S. Sanghera.** 1992. MAP kinases: charting the regulatory pathways. Science **257:**1355–1356.
45. **Perrotti, N., D. Accili, B. Marcus Samuels, R. W. Rees Jones, and S. I. Taylor.** 1987. Insulin stimulates phosphorylation of a 120-kDa glycoprotein substrate (pp120) for the receptor-associated protein kinase in intact H-35 hepatoma cells. Proc. Natl. Acad. Sci. USA **84:**3137–3140.
46. **Piccione, E., R. Case, S. M. Domchek, P. Hu, M. Chaudhuri, J. M. Backer, J. Schlessinger, and S. E. Shoelson.** 1993. Phosphatidylinositol 3-kinase p85 SH2 domain specificity defined by direct phosphopeptide/SH2 domain binding. Biochemistry **32:**3197–3202.
47. **Rice, K. M., G. E. Leinhard, and C. W. Garner.** 1992. Regulation of the expression of pp160, a putative insulin receptor signal protein, by insulin, dexamethasone, and 1-methyl-3-isobutylxanthine in 3T3-L1 adipocytes. J. Biol. Chem. **267:**10163–10167.
48. **Roberts, T. M.** 1992. A signal chain of events. Nature (London) **360:**534–535.
49. **Rosen, O. M.** 1987. After insulin binds. Science **237:**1452–1458.
50. **Rothenberg, P. L., W. S. Lane, A. Karasik, J. Backer, M. F. White, and C. R. Kahn.** 1991. Purification and partial sequence analysis of pp185, the major cellular substrate of the insulin receptor tyrosine kinase. J. Biol. Chem. **266:**8302–8311.
51. **Ruderman, N., R. Kapeller, M. F. White, and L. C. Cantley.** 1990. Activation of phosphatidylinositol 3-kinase by insulin. Proc. Natl. Acad. Sci. USA **87:**1411–1415.
52. **Saad, M. J. A., E. Araki, M. Miralpeix, P. L. Rothenberg, M. F. White, and C. R. Kahn.** 1992. Regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance. J. Clin. Invest. **90:**1839–1849.
53. **Shoelson, S. E., M. Sivaraja, K. P. Williams, P. Hu, J. Schlessinger, and M. A. Weiss.** 1993. Specific phosphopeptide binding regulates a conformational change in the PI 3-kinase SH2 domain associated with enzyme activation. EMBO J. **12:**795–802.
54. **Sjolander, A., K. Yamamoto, B. E. Huber, and E. G. Lapetina.** 1991. Association of p21^{ras} with phosphatidylinositol 3-kinase. Proc. Natl. Acad. Sci. USA **88:**7908–7912.
55. **Skolnik, E. Y., B. Margolis, M. Mohammadi, E. Lowenstein, R. Fischer, A. Drepps, A. Ullrich, and J. Schlessinger.** 1991. Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell **65:**83–90.
56. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene **67:**31–40.
57. **Smith, L. D.** 1989. The induction of oocyte maturation: transmembrane signaling events and regulation of the cell cycle. Development **107:**685–699.
58. **Sturgill, T. W., and J. Wu.** 1991. Recent progress in characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. Biochim. Biophys. Acta **1092:**350–357.
59. **Sun, X. J., M. Miralpeix, M. G. Myers, Jr., E. M. Glasheen, J. M. Backer, C. R. Kahn, and M. F. White.** 1992. Expression and function of IRS-1 in insulin signal transmission. J. Biol. Chem. **267:**22662–22672.
60. **Sun, X. J., P. Rothenberg, C. R. Kahn, J. M. Backer, E. Araki, P. A. Wilden, D. A. Cahill, B. J. Goldstein, and M. F. White.** 1991. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature (London) **352:**73–77.
61. **Ullrich, A., and J. Schlessinger.** 1990. Signal transduction by receptors with tyrosine kinase activity. Cell **61:**203–211.
62. **Vera, J. C., and O. M. Rosen.** 1990. Reconstitution of an insulin signaling pathway in *Xenopus laevis* oocytes: coexpression of a mammalian insulin receptor and three different mammalian hexose transporters. Mol. Cell. Biol. **10:**743–751.
63. **White, M. F., and C. R. Kahn.** 1986. The insulin receptor and tyrosine phosphorylation, p. 247–302. *In* P. D. Boyer and E. G. Krebs (ed.), The enzymes. Academic Press, Inc., Orlando, Fla.
64. **White, M. F., R. Maron, and C. R. Kahn.** 1985. Insulin rapidly stimulates tyrosine phosphorylation of a Mr 185,000 protein in intact cells. Nature (London) **318:**183–186.
65. **Wilden, P. A., K. Siddle, E. Haring, J. M. Backer, M. F. White, and C. R. Kahn.** 1992. The role of insulin receptor kinase domain autophosphorylation in receptor-mediated activities. Analysis with insulin and anti-receptor antibodies. J. Biol. Chem. **267:**13719–13727.
66. **Yonezawa, Y., H. Ueda, K. Hara, K. Nishida, A. Ando, A. Charanieu, H. Matsuba, K. Shii, K. Yokono, Y. Fukui, B. Calas, G. Grigorescu, R. Dhand, I. Gout, M. Otsu, M. D. Waterfield, and M. Kasuga.** 1992. Insulin-dependent formation of a complex containing an 85-kDa subunit of phosphatidylinositol 3-kinase and tyrosine-phosphorylated insulin receptor substrate 1. J. Biol. Chem. **267:**25985–25966.