Expression of the Insulin Receptor with a Recombinant Vaccinia Virus

BIOCHEMICAL EVIDENCE THAT THE INSULIN RECEPTOR HAS INTRINSIC SERINE KINASE ACTIVITY*

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We have previously reported the tight association of a serine kinase activity with the human insulin receptor (Lewis, R. E., Wu, G. P., MacDonald, R. G., and Czech, M. P. (1990) J. Biol. Chem. 265, 947–954). We tested the possibility that the associated serine kinase activity was intrinsic to the receptor catalytic domain. The ratio of phosphoserine to phosphotyrosine on insulin receptors phosphorylated in vitro was used as an index of the associated serine kinase activity. Phosphorylation and phosphoamino acid analysis of insulin proreceptors revealed associated serine kinase activity early in receptor synthesis. Insulin receptors were expressed in HeLa cells using a recombinant vaccinia virus. The ratio of phosphoserine to phosphotyrosine on insulin receptors expressed by the recombinant vaccinia virus was determined relative to endogenous insulin receptors in cells treated with α-amanitin to block host cell mRNA synthesis. α-Amanitin treatment had no effect on the ratio of phosphoserine to phosphotyrosine on insulin receptors expressed from the recombinant virus even though they were present in a 4000-fold excess above endogenous receptors. We conclude that the serine kinase activity associated with the insulin receptor is intrinsic to the receptor catalytic domain. Receptor-catalyzed autophosphorylation of serine may play an important role in modulating insulin receptor signaling.

Insulin-stimulated phosphorylation of the insulin receptor cytoplasmic domain plays a central role in the transmission and regulation of insulin receptor signal transduction. Insulin activation of the receptor tyrosine kinase results in a cascade of tyrosine autophosphorylation. Autophosphorylation of tyrosines 1158, 1162, and 1163 maintains receptor kinase activation in the absence of bound insulin (1, 2). Phosphorylation of tyrosines 1328 and 1334 in the receptor carboxyl-terminal tail occurs upon activation of the insulin receptor tyrosine kinase (3–5). Serine phosphorylation of the insulin receptor cytoplasmic domain also occurs following insulin activation but subsequent to receptor tyrosine autophosphorylation (6, 7). Phorbol ester or forskolin addition to cells has been reported to stimulate the serine phosphorylation of the insulin receptor β subunit (8–10). A decrease in insulin receptor tyrosine kinase activity coincides with the phosphorylation of insulin receptors by these agents (10, 11). Protein kinase C (12) and cyclic AMP-dependent protein kinase (13) are each capable of phosphorylating the insulin receptor in cell-free systems. Receptor phosphorylation catalyzed by each of these kinases results in a decrease in insulin-stimulated tyrosine kinase activity toward exogenous substrates (12, 13). Additional studies with kinase-inactive insulin receptor mutants indicate that the receptor tyrosine kinase is important for the complete biological activity of insulin. Consequently, inhibition of receptor tyrosine kinase activity by serine phosphorylation may be an important mechanism for regulating receptor signaling in intact cells.

An insulin-sensitive serine kinase activity is associated with the insulin receptor in eluates from wheat germ-agglutinin affinity columns (14–16). This insulin-stimulated serine kinase activity remains tightly associated with highly purified insulin receptors eluted from insulin-agarose affinity columns (17, 18). The tightly associated serine kinase activity is also capable of phosphorylating highly purified insulin receptors on serine and threonine sites within the cytoplasmic domain that are also phosphorylated in intact cells (17). The serine kinase activity tightly associated with the insulin receptor phosphorylates synthetic peptides identical to sites of insulin receptor serine and threonine phosphorylation in vivo. The conclusion that the insulin receptor activates the receptor-associated serine kinase is supported by the observation that peptide phosphorylation is enhanced by insulin addition to the affinity-purified receptor preparations (17).

The presence of serine kinase activity associated with insulin receptors immunoprecipitated from Sf9 cells infected with a recombinant baculovirus containing the human insulin receptor cDNA has been reported (6). Although these receptor preparations contain primarily unprocessed proreceptor that is unresponsive to insulin activation, they suggest the possibility that the insulin receptor may contain intrinsic serine/threonine as well as tyrosine kinase activity. Consistent with this possibility, the tyrosine kinase inhibitor (hydroxy-2-naphthalenylmethyl)phosphonic acid blocks the ability of insulin receptor preparations from baculovirus-infected Sf9 cells containing associated serine kinase activity from phosphorylating a synthetic peptide containing an insulin receptor serine phosphorylation site (6).

Biochemical analysis has provided evidence for the existence of several “dual specificity” kinases (19). The amino acid sequences of those kinases considered to be capable of phosphorylation on tyrosine, serine, and threonine most closely resemble the family of serinethreonine kinases. We tested the hypothesis that the insulin receptor kinase had the capability of phosphorylating its cytoplasmic domain on serine and tyrosine. We expressed the insulin receptor under control of the bacteriophage T7 promoter in HeLa cells that were also in-
fected with a recombinant vaccinia virus that expresses T7 polymerase. Receptor-associated serine kinase activity can be detected in insulin proreceptors early in synthesis. Furthermore, we demonstrate that the serine kinase activity associated with insulin receptors expressed under control of the T7 promoter is not altered in receptor preparations isolated under conditions that block synthesis of host cell mRNA.

EXPERIMENTAL PROCEDURES

Materials—Antibodies CT-1 and 83–14 were gracious gifts from Ken Siddle (Cambridge, UK). Lentil lectin-agarose and wheat germ agglu- tinin agarose were obtained from E.Y. Labs, Inc. (Edison, NJ) and Nco I was from New England Biolabs, Inc., insulin was from Calbiochem, and a-amanitin and phosphoamino acid standards were from Sigma. Thin layer chromatography plates used for phosphoamino acid analysis were obtained from Machery-Nagel. Cell culture and COS-1 cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum. All reagents were from Sigma.

Phosphoamino Acid Analysis—Phosphoamino acid analysis was performed as described (17). The 32P-labeled insulin receptor β subunit was localized by autoradiography and excised. The labeled bands were counted for Cerenkov radiation and washed alternately in acetone and water three times for 10 min each time. Gel fragments were placed in a 1.5-ml Eppendorf tube with 0.3 ml of 0.25% ammonium bicarbonate, pH 8.2, containing 30 μg/ml trypsin and digested at 37°C for 18 h. The tryptic elute containing β subunit phosphopeptides was removed and lyophilized to dryness in a clean 1.5-ml Eppendorf tube. Dried phosphopeptides were reconstituted in 0.2 ml of 6 N HCl. Partial hydrolysis of the phosphopeptides was performed at 110°C for 1 h, after which the samples were dialyzed against 20 ml of water. Lyophilized samples were then reconstituted in 0.1 ml of 100 μl of water, and lyophilized a second time. The entire contents of each tube were spotted on a 20 × 20-cm thin layer cellulose plate in 5 μl of 30% formic acid containing 1 mg/ml of each phosphoamino acid standard. The plates were visualized with ninhydrin. Radiolabeled phosphoamino acids were identified by autoradiography. The relative amounts of phosphopeptide incorporated into tyrosine or serine were determined on a Betagen Betascope and a Molecular Dynamics PhosphorImager.

High Pressure Liquid Chromatography Phosphopeptide Mapping—For phosphopeptide mapping, the phosphorylated insulin receptor was separated on an 8% SDS-polyacrylamide gel. The insulin receptor β subunit was localized by autoradiography and digested twice with 100 μg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin in 0.25% ammonium bicarbonate. Phosphopeptides were separated on a Brownlee Aquapore OD300 column with a gradient of 50% acetonitrile in 0.1% trifluoroacetic acid at 0.25 ml/min. Fractions were collected at 30-s intervals and counted for Cerenkov radiation. Recovery of 32P was 70–84%.

Two-dimensional Peptide Mapping—HPLC2 phosphopeptide peaks were further resolved in two dimensions on cellulose thin layer plates. HPLC fractions were pooled, lyophilized, reconstituted in 5 μl of pH 1.9 buffer, and spotted on thin layer plates. The tracking dye e-2,4-dinitrophenyl lysine was spotted adjacent to radioactive samples. The samples were separated by electrophoresis at 1900 V for 35 min in pH 1.9 buffer. After electrophoresis each plate was allowed to dry thoroughly, and then chromatography was performed at a right angle to the direction of electrophoresis in n-butanol/pyridine/acetic acid/water (15:10:3:12) as described previously (17). After chromatography the plates were dried and exposed to x-ray film (XAR-5, Kodak) to localize 32P-labeled phosphopeptides.

Lentil Lectin Chromatography—Infected or transfected HeLa cells were lysed in 1 ml of 0.1 M Tris, pH 7.4, and concentrated 10-fold with Buffer A containing 0.1% Triton X-100 and 0.3 M α-methylmannopyranoside.
The purpose of this study was to test whether insulin receptor-associated serine kinase activity is intrinsic to the receptor. To test this possibility, human insulin receptor was initially expressed in COS-1 cells. Extracts from transfected cells were partially purified on wheat germ agglutinin-agarose. The flow-through fraction from the wheat germ agglutinin-agarose was approximately 15% of the phosphotyrosine released by hydrolysis from these phosphoproteins (Table I). Similarly, phosphoserine detected in proreceptor and mature insulin receptor eluates was approximately 15% of detectable phosphotyrosine (Table I).

The data in Fig. 1 suggest that serine kinase activity becomes associated with the insulin receptor early in its biosynthetic pathway. The level of serine phosphorylation detected with the high mannose form of the insulin receptor precursor appears comparable to that co-precipitating with biosynthetically mature forms. These observations suggest that either a distinct serine kinase associates with insulin receptor precursors early in synthesis or the insulin receptor tyrosine kinase has the intrinsic ability to phosphorylate itself on serine residues. To distinguish between these two possibilities, we developed a system to express the insulin receptor in cells in which endogenous mRNA synthesis had been blocked for prolonged periods of time. We subcloned the insulin receptor cDNA into the transfer vector pTM-1 (21) to create the plasmid pTM1hIR. This construct contains a T7 promoter and 5' untranslated sequences from encephalomyocarditis virus for efficient transcription and translation of uncapped insulin receptor mRNA. Insulin receptors were detected by immune complex kinase assay of insulin receptor immunoprecipitates from HeLa cells transfected with pTM1hIR and infected with a recombinant vaccinia virus (VTF7) encoding T7 polymerase (Fig. 2A, lane 5). To determine the amount of endogenous insulin receptor contributed by HeLa cells, control infections and transfections were performed. Similar amounts of phosphorylated insulin receptor precursor and insulin receptor β subunit were detected in the immune complex from HeLa cells infected with wild-type vaccinia virus (WR) and transfected with recombinant plasmid pTM1hIR (Fig. 2A, lane 1) or when infected with the recombinant virus VTF7 and transfected with the control phosphoserine (Fig. 1B, left panel). Interestingly, serine phosphorylation was also detected in high mannose insulin receptor precursors (Fig. 1B, right panel). Trace amounts of phosphotheosine could also be detected in receptor precursors immunopurified from wheat germ agglutinin-agarose or lentil lectin-agarose eluates (Fig. 1B). The phosphoserine content of receptor precursor and β subunit immunopurified from wheat germ agglutinin-agarose was 11–12% of the phosphotyrosine released by hydrolysis from these phosphoproteins (Table I). Similarly, phosphoserine detected in proreceptor from lentil lectin-agarose eluates was approximately 15% of detectable phosphotyrosine (Table I).

Insulin Receptor Kinase Activity

**TABLE I**

| Transient expression in COS-1 cells | Ratio of 32P incorporation (phosphotyrosine/phosphoserine) |
|------------------------------------|---------------------------------------------------------|
| Wheat germ agglutinin-agarose     |                                                         |
| Proreceptor                        | 8.5:1                                                   |
| β subunit                          | 9.2:1                                                   |
| Lentil lectin-agarose              |                                                         |
| Proreceptor                        | 6.8:1                                                   |
| Vaccinia-mediated expression       |                                                         |
| pTM1hIR + WR                       |                                                         |
| Proreceptor                        | 6.0:1                                                   |
| β subunit                          | 5.9:1                                                   |
| pTM1-1 + VTF7                      |                                                         |
| Proreceptor                        | 4.7:1                                                   |
| β subunit                          | 5.1:1                                                   |
| pTM1hIR + VTF7                     |                                                         |
| Proreceptor                        | 6.4:1                                                   |
| β subunit                          | 5.6:1                                                   |
| pTM1hIR + VTF7 + α-amanitin        |                                                         |
| Proreceptor                        | 5.9:1                                                   |
| β subunit                          | 7.1:1                                                   |

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Insulin receptor synthesis (Fig. 2)

Endogenous HeLa mRNA by the addition of the RNA polymerase inhibitor α-amanitin (29). Treatment of control cells with 10 μg/ml α-amanitin for 18 h prior to lysis blocked endogenous insulin receptor synthesis (Fig. 2A, lanes 3 and 4). α-Amanitin had no effect, however, on the level of insulin receptor expressed in cells infected with VTF7 and transfected with pTM1hIR (Fig. 2A, lane 6). In multiple experiments insulin receptors expressed in VTF7-infected and pTM1hIR-transfected cells were 8–140-fold more abundant than endogenous insulin receptors from control cells.

Phosphoamino acid analysis was performed on insulin receptors from control-treated HeLa cells and from HeLa cells infected with VTF7 and transfected with pTM1hIR. Quantitative analysis of the 32P incorporation on tyrosine and serine in the insulin receptor β subunit demonstrated that treatment with α-amanitin has no effect on the relative amount of serine phosphorylation of insulin receptors expressed from T7 polymerase-generated transcripts (Fig. 2B and Table I). Thus, serine kinase activity remained associated with the insulin receptors synthesized from T7 polymerase-generated transcripts even though endogenous mRNA synthesis was inhibited.

The efficiency of insulin receptor production was improved by the construction and co-infection of a recombinant vaccinia virus, VTF7hIR, that contained the human insulin receptor cDNA under control of the T7 promoter. Co-infection of HeLa cells with VTF7 and VTF7hIR followed by treatment with α-amanitin resulted in a 4000-fold increase in mature, phosphorylated insulin receptors over the level of receptor observed in α-amanitin-treated cells infected with VTF7 and the control virus WR (Fig. 3A and B). Phosphoamino acid analysis of 32P-labeled insulin receptors revealed that phosphoserine persisted in both the proreceptor and mature β subunits produced in VTF7hIR-infected and α-amanitin-treated cells (Fig. 3C). Quantitative analysis of phosphoamino acids present in the 32P-labeled insulin receptors produced in VTF7hIR-infected and α-amanitin-treated cells revealed phosphotyrosine:phosphothreonine ratios of 6.3 and 10.7 in the proreceptors and mature β subunits, respectively. These ratios are comparable to those seen at lower levels of insulin receptor expression in COS-1 cells and in pTM1hIR-transfected HeLa cells (Fig. 4 and Table I).

Phosphopeptide mapping was performed to determine if treatments that inhibited the synthesis of endogenous insulin receptors specifically altered the phosphorylation of individual sites within the insulin receptor cytoplasmic domain. HeLa cells co-infected with VTF7 and VTF7hIR were treated with α-amanitin or left untreated. Insulin receptors were immunoprecipitated, labeled with [γ-32P]ATP in the presence of 100 nM insulin, and resolved by SDS-polyacrylamide gel electrophoresis. 32P-labeled receptor β subunits were localized by autoradiography and digested with trypsin. The resulting phos-
Serine and threonine phosphorylation of insulin receptors expressed at different levels in transfected or virus-infected cells. Insulin receptors expressed in HeLa cells were immunoprecipitated and phosphorylated in vitro with [γ-32P]ATP. Insulin proreceptors (lanes 1–7) and insulin receptor β subunits (lanes 8–12) were isolated and phosphorylated from HeLa cells transfected with plasmids pTM1hIR (lanes 1, 3, 5, 6, 8, 10, and 11) or control plasmid pTM-1 (lanes 2, 4, and 9) and infected with the wild-type control vaccinia virus VTF7 (lanes 2, 4, 5, 6, 9, 10, and 11). Insulin receptor expression was also generated by co-infection with the recombinant viruses VTF7hIR and VTF7 (lanes 7 and 12). Following transfection and infection procedures, cells were incubated with (lanes 3, 4, 6, 7, 11, and 12) or without (lanes 1, 2, 5, 8, 9, and 10) α-amanitin for 18 h. 32P-labeled insulin receptor levels in proreceptors (lanes 2–6) and in β subunits (lanes 9–11) were normalized to control treatments in lanes 1 and 8, respectively. 32P-labeled insulin receptor levels indicated in lanes 7 and 12 were normalized relative to receptor expression in cells co-infected with control viruses WR and VTF7 (Fig. 3). The ratio of phosphorylase to phosphothreonine was determined from phosphoamino acid analysis of each receptor sample and is indicated as an open circle. The presence of multiple circles denotes additional experiments for that condition.

Two-dimensional analysis revealed that all phosphopeptides were conserved between insulin receptors isolated from α-amanitin-treated and untreated cells. Furthermore, three phosphopeptides within HPLC peak 2 contained phosphoserine and were conserved between α-amanitin-treated and untreated cells (Fig. 5, B and C). All other phosphopeptides resolved in each HPLC peak contained only phosphotyrosine (data not shown). These data demonstrate that although α-amanitin treatment blocks endogenous insulin receptor expression, it has no effect on the ability of over-expressed insulin receptors to be phosphorylated on specific serine phosphorylation sites in vitro.

We investigated the nature of the insulin receptor-associated serine kinase activity by examining its temporal association with the insulin receptor during synthesis. Our observations indicate that insulin receptor serine kinase activity is detectable in insulin receptors at early stages of receptor synthesis and persists during treatments that block endogenous RNA expression. Serine kinase activity was detected in high mannose forms of the insulin proreceptor, a form of the receptor found in the endoplasmic reticulum and medial Golgi (26, 27). The extent of proreceptor serine phosphorylation was comparable to that associated with fully processed receptor β subunits (Fig. 1B and Table I). If a distinct serine kinase associates with the insulin receptor, this observation suggests that it must do so prior to maturation of carbohydrate side chains and proteolytic processing of receptor subunits.

We also determined whether serine kinase activity would remain associated with insulin receptors translated from receptor mRNA transcripts generated by T7 polymerase in cells treated with and without 10 μg/ml α-amanitin for 18 h. α-Amanitin blocks de novo mRNA synthesis by inhibiting RNA polymerase II (29). α-Amanitin has no effect however, on T7 polymerase activity. Thus, α-amanitin treatment of cells infected with VTF7 and transfected with pTM1hIR will yield insulin receptors produced only by T7 polymerase provided by the recombinant virus (Fig. 2A, compare lanes 5 and 6). Insulin receptors expressed in VTF7-infected and pTM1hIR-transfected cells were at least 8-fold more abundant than insulin receptors in control cells (note times of exposure for lanes 5 and 6 versus lanes 1–4 in Fig. 2A).

Insulin receptor levels were elevated greatly with a recombinant vaccinia virus, VTF7hIR, encoding the human insulin receptor under control of the T7 promoter. Co-infection of VTF7hIR with the virus VTF7 allowed T7-mediated expression of insulin receptors in α-amanitin-treated cells that was 4000-fold above endogenous receptor expression in α-amanitin cells infected with control virus (Fig. 3). Despite the ability of the insulin proreceptor to be phosphorylated on specific serine phosphorylation sites, it is not associated with a serine kinase activity that is detectable in the α-amanitin-treated cells. The reason for this is unknown, but could be related to the fact that the insulin receptor under control of the T7 promoter encodes an inactive receptor subunit (30).

### Discussion

We determined whether serine kinase activity would remain associated with insulin receptors translated from receptor mRNA transcripts generated by T7 polymerase in cells treated with and without 10 μg/ml α-amanitin for 18 h. α-Amanitin blocks de novo mRNA synthesis by inhibiting RNA polymerase II (29). α-Amanitin has no effect however, on T7 polymerase activity. Thus, α-amanitin treatment of cells infected with VTF7 and transfected with pTM1hIR will yield insulin receptors produced only by T7 polymerase provided by the recombinant virus (Fig. 2A, compare lanes 5 and 6). Insulin receptors expressed in VTF7-infected and pTM1hIR-transfected cells were at least 8-fold more abundant than insulin receptors in control cells (note times of exposure for lanes 5 and 6 versus lanes 1–4 in Fig. 2A).

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\(a\)-amanitin to block endogenous insulin receptor production, VTF7hIR-generated insulin receptors retained serine kinase activity comparable to that of endogenous insulin receptors isolated from control-treated cells (Figs. 3C and 4). The fact that the amount of serine phosphate on the insulin receptor precursor and mature \(\beta\) subunit does not change appreciably in cells over-expressing the receptor could be explained by the presence of an excess amount of an associating serine kinase. However, persistence of insulin receptor-associated serine kinase activity of VTF7hIR-infected cells treated with \(a\)-amanitin for 18 h would mean that a distinct serine kinase activity would also have an extremely slow turnover rate compared with the endogenous insulin receptors that were abolished by \(a\)-amanitin. Consequently, we believe a more likely explanation for the insulin receptor-associated serine kinase is that the kinase domain of the insulin receptor \(\beta\) subunit has the intrinsic ability to transfer phosphate to serine as well as tyrosine. Phosphopeptide mapping and phosphoamino acid analysis (Fig. 5) demonstrated that the pattern of serine phosphorylation on insulin receptors in VTF7hIR-infected cells is not altered by \(a\)-amanitin treatment. Thus, the serine kinase activity associated with the insulin receptor would not appear to be composed of multiple activities both distinct and intrinsic to the insulin receptor catalytic domain.

Previous reports have demonstrated that an insulin-sensitive serine kinase activity was associated with human insulin receptors partially purified from human placental membranes on wheat germ agglutinin-agarose (14–17). An associated serine kinase is reportedly dissociated from the insulin receptor by 1 M NaCl (15, 30), and that replacement of the NaCl eluate from kinases partially purified from human placental membranes with a serine kinase activity was associated with human insulin receptors. The associated serine kinase activity was not capable of phosphorylation on sites within the juxtamembrane domain of the insulin receptor catalytic domain.

The predicted amino acid sequences of cloned kinase genes have been suggested to contain information predictive of their catalytic specificity (19). The insulin receptor, however, shares greater homology with protein tyrosine kinases than with dual specificity kinases. This difference demonstrates the importance of directly determining phosphoacceptor specificity.

Phosphorylation of the insulin receptor by the associated serine kinase occurs on sites within the juxtamembrane domain (31, 32) and the carboxyl-terminal tail (17). Phosphopeptide maps suggest that as many as four additional serine phosphoacceptor sites for the receptor-associated serine kinase may exist (17). Determination of the role of serine autophosphorylation in the enzymatic function of the insulin receptor and in insulin-mediated control of cellular metabolism may require identification of these sites. Analysis of mutant receptors lacking all serine phosphorylation sites may be necessary to evoke an effect on receptor enzymatic activity and signaling.

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REFERENCES

1. Ellis, L., Clauser, E., Morgan, D. O., Edery, M., Roth, R. A., and Rutter, W. J. (1986) Cell 45, 721–732
2. Yonezawa, K., and Roth, R. A. (1991) Mol. Endocrinol. 5, 194–200
3. Tornqvist, H. E., Pierce, A. R., Fradkleton, A. R., Nemenoff, R. A., and Avruch, J. (1987) J. Biol. Chem. 262, 10212–10219
4. Kohnaski, R. A. (1993) Biochemistry 32, 5773–5780
5. Baltensperger, K., Lewis, R. E., Woon, C.-W., Vissavajhala, P., Ross, A. H., and Czech, M. P. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7885–7889
6. Pang, D. T., Sharma, B. R., Shafii, J. A., White, M. F., and Kahn, C. R. (1985) J. Biol. Chem. 260, 7131–7136
7. Jacob, S., Sanyoum, N. E., Sattel, A. R., and Cuatrecasas, P. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 6211–6213
8. Takayama, S., White, M. F., Laurits, V., and Kahn, C. R. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 7797–7801
9. Stadtmueller, L., and Rosen, O. M. (1986) J. Biol. Chem. 261, 3402–3407
10. Takayama, S., White, M. F., Laurits, V., and Kahn, C. R. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 7797–7801
11. Bollag, G. E., Roth, R. A., Beauvoin, J., Mochly-Rosen, D., and Koshland, D. E., Jr. (1983) Biochemistry 22, 5021–5027
12. Roth, R. A., and Beauvoin, J. (1987) Diabetes 36, 123–126
13. Zick, Y., Grunberger, G., Podskalny, J. M., Moncada, V., Taylor, S. L., Gorden, P., and Roth, J. (1983) Biochem. Biophys. Res. Commun. 116, 1129–1135
14. Smith, D. M., and Sale, G. J. (1988) Biochem. J. 256, 903–909
15. Ballott, R., Kowalski, A., Le Marchand-Brustel, Y., and Van Obberghen, E. (1986) Biochem. Biophys. Res. Commun. 139, 179–185
16. Lewis, R. E., Wu, G. P., MacDonald, R. G., and Czech, M. P. (1990) J. Biol. Chem. 265, 947–954
17. Heidenreich, K., Pausduske, M., Molders, M., and Klein, H. W. (1994) Biol. Chem. Hoppe-Seyer 375, 99–104
18. Lindberg, R. A., Quinn, A. M., and Hunter, T. (1992 Trends Biochem. Sci. 17, 114–119
19. Whitaker, J., Okamoto, A. K., Thys, R., Beli, G. I., Steiner, D. F., and Hidmn, C. A. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 5237–5241
20. Moss, B., Eltrey-stein, O., Mizukami, T., Alexander, W. A., and Fuerst, T. R. (1990) Nature 348, 91–92
21. Fuerst, T. R., Earl, P. L., and Moss, B. (1987) Mol. Cell. Biol. 7, 2538–2544
22. Chave, C., and Okawama, H. (1987) Mol. Cell. Biol. 7, 2743–2752
23. Ganderon, R. H., Stanley, K. K., Field, C. E., Coghlan, M. P., Soos, M. A., and Siddiqui, K. (1992) J. Biochem. 288, 195–206
24. Soos, M. A., Siddiqui, K., Baron, M. D., Heuchard, J. M., Luzio, J. P., Bellati, J., and Lennox, E. S. (1988) Biochem. J. 256, 190–208
25. Olson, T. S., and Lane, M. D. (1987) J. Biol. Chem. 262, 6816–6822
26. Olson, T. S., Bamberger, M. J., and Lane, M. D. (1988) J. Biol. Chem. 263, 4722–4731
27. Leconte, I., Auzan, C., Debart, A., Bossi, R., and Clauser, E. (1992) Trends Biochem. Sci. 17, 149–154
28. Boldogh, I., Abu Bakar, S., Fons, M. P., Deng, C. Z., and Albrecht, T. (1991) J. Cell. Biol. 139, 179–185
29. Leconte, I., Auzan, C., Debart, A., Bossi, R., and Clauser, E. (1992) Trends Biochem. Sci. 17, 149–154
30. Boldogh, I., Abu Bakar, S., Fons, M. P., Deng, C. Z., and Albrecht, T. (1991) J. Cell. Biol. 139, 179–185
31. Asano, K. A., Atkinson, P. G. P., Carter, W. G., and Sales, G. J. (1995) Biochem. J. 308, 915–922
32. Fehlner, P. E., Backer, J. M., Ting, G. L., Wilden, P. A., Sun, X. J., Kahn, R., and White, M. F. (1993) J. Biol. Chem. 268, 11256–11264
33. Liu, F., and Roth, R. A. (1994) Biochem. J. 298, 471–477
34. Ebina, Y., Ellis, L., Jarsagam, K., Edery, M., Graff, L., Clauser, E., Ou, J.-H., Masarz, F., Kan, Y.-W., Goldfine, I. D., Roth, R. A., and Rutter, W. J. (1985) Cell 40, 747–758
