A Phosphatidylinositol 3-Kinase-independent Insulin Signaling Pathway to N-WASP/Arp2/3/F-actin Required for GLUT4 Glucose Transporter Recycling

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Recruitment of intracellular glucose transporter 4 (GLUT4) to the plasma membrane of fat and muscle cells in response to insulin requires phosphatidylinositol (PI) 3-kinase as well as a proposed PI 3-kinase-independent pathway leading to activation of the small GTPase TC10. Here we show that in cultured adipocytes insulin causes acute cortical localization of the actin-regulatory neural Wiskott-Aldrich syndrome protein (N-WASP) and actin-related protein-3 (Arp3) as well as cortical F-actin polymerization by a mechanism that is insensitive to the PI 3-kinase inhibitor wortmannin. Expression of the dominant inhibitory N-WASP-ΔWA protein lacking the Arp and actin binding regions attenuates the cortical F-actin rearrangements by insulin in these cells. Remarkably, the N-WASP-ΔWA protein also inhibits insulin action on GLUT4 translocation, indicating dependence of GLUT4 recycling on N-WASP-directed cortical F-actin assembly. TC10 exhibits sequence similarity to Cdc42 and has been reported to bind N-WASP. We show the inhibitory TC10 (T31N) mutant, which abrogates insulin-stimulated GLUT4 translocation and glucose transport, also inhibits both cortical localization of N-WASP and F-actin formation in response to insulin. These findings reveal that N-WASP likely functions downstream of TC10 in a PI 3-kinase-independent insulin signaling pathway to mobilize cortical F-actin, which in turn promotes GLUT4 responsiveness to insulin.

Insulin stimulates glucose uptake by skeletal muscle and adipose tissues primarily through regulation of the subcellular distribution of the glucose transporter 4 (GLUT4)1 (1, 2). In response to insulin, a fraction of GLUT4 present in intracellular membranes is redistributed to the plasma membrane, resulting in an increase of GLUT4 on the cell surface and enhanced glucose uptake by these cells. This effect of insulin is important in maintaining glucose homeostasis in humans, and impaired insulin action can contribute to the pathogenesis of type 2 diabetes (3). The precise mechanism by which insulin directs exocytosis of GLUT4-containing membrane vesicles remains obscure. However, it is established that activation of the insulin receptor tyrosine kinase catalyzes tyrosine phosphorylation of insulin receptor substrate proteins that bind to Src-homology 2 domain-containing molecules, including the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase) (4, 5). This results in activation of the p110 catalytic subunit of the kinase, which then phosphorylates cellular polyphosphoinositides at the D-3 position, forming signaling molecules such as phosphatidylinositol 3,4,5-trisphosphate. Multiple studies using various pharmacologic inhibitors, overexpression of constitutively active or dominant negative mutants, and microinjection of blocking antibodies have suggested a necessary role of the p85/p110-type PI 3-kinase in insulin-stimulated GLUT4 translocation and glucose transport (1, 2, 6–8).

On the other hand, several lines of evidence suggest the requirement of PI 3-kinase-independent pathway(s) in GLUT4 translocation and glucose transport regulation. For example, treatment of cultured adipocytes with a cell-permeable analog of phosphatidylinositol 3,4,5-trisphosphate failed to mimic the action of insulin on hexose transport (9). Furthermore, interleukin-4 and anti-integrin antibodies do not apparently stimulate glucose transport in adipocytes, although these agonists activate PI 3-kinase (10, 11). Other studies suggest that both endothelin-1 and physical exercise stimulate glucose uptake independent of PI 3-kinase activation in cultured adipocytes and muscle, respectively (12–15). Recent reports indicate that insulin stimulation of GLUT4 translocation requires activation of the small GTPase TC10 through a PI 3-kinase-independent pathway involving the docking of adapter protein CAP and tyrosine phosphorylation of c-Cbl in adipocytes (16, 17). The link between TC10 activation and GLUT4 translocation is unresolved (17). Thus, although PI 3-kinase activation and 3′-polyphosphoinositide production appears necessary for insulin to act on glucose transport, other signaling pathway(s) may also be required for optimal GLUT4 regulation by the hormone.

In searching for such PI 3-kinase-independent signaling elements that may be downstream of TC10, we focused on recent findings from our laboratory (13, 18, 19) and others (20–26) linking the microtubule- and actin-based cytoskeleton to recycling of GLUT4-containing vesicles. In particular, insulin elicits its actin filament (F-actin) formation (13, 18–24, 27, 28), and reagents that cause actin depolymerization inhibit insulin-induced GLUT4 translocation and glucose uptake (18–24). Thus insulin signaling to polymerize cortical F-actin apparently represents a required pathway for optimal movement or fusion of GLUT4-containing membranes to the cell surface membrane. The present study was designed to test whether this signaling pathway may represent the PI 3-kinase-independent cascade involving the TC10 GTPase proposed to be necessary for insu-
lin action on GLUT4 (17). We report here that acute insulin signaling causes cortical actin polymerization in 3T3-L1 adipocytes secondary to the recruitment of the TC10-interacting protein neural Wiskott-Aldrich syndrome protein (N-WASP) to the cortical regions of these cells. These effects are unaffected by the PI 3-kinase inhibitor wortmannin. N-WASP in turn is known to bind the actin-related protein-2/3 (Arp2/3) complex, which causes actin nucleation and polymerization (29, 30). We find that dominant negative TC10 (T31N) blocks insulin signaling to N-WASP, while the inhibitory constructs TC10 (T31N) and N-WASP-ΔWA attenuate insulin signaling to both cortical F-actin and GLUT4 translocation to the plasma membrane. These data reveal a PI 3-kinase-independent signaling pathway from the insulin receptor to cortical actin polymerization that is required for optimal GLUT4 glucose transporter regulation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human insulin was obtained from Eli Lilly Co. Rabbit polyclonal anti-GLUT4 antibody was raised against the COOH-terminal 12 amino acids. Rabbit polyclonal anti-rat N-WASP antibody was purchased from Sigma (St. Louis, MO). Goat polyclonal anti-human Arp3 antibody and mouse monoclonal anti-Myc epitope antibody clone 9E10 were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-HA epitope antibody was produced in this laboratory (33). The rhodamine- or fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse and anti-rabbit antibodies were from BIOSOURCE International (Camarillo, CA). FITC-conjugated donkey anti-goat IgG (Jackson ImmunoResearch (West Grove, PA)). Rhodamine-conjugated phallolidin and goat anti-rabbit IgG conjugated with Alexa-350 were from Molecular Probes, Inc. (Eugene, OR).

**cDNA Expression Constructs**—To construct Myc-GLUT4-EGFP, a site-directed mutagenesis was performed in the GLUT4 cDNA by inserting DNA sequence encoding Myc epitope (EQLISEEDLLK) between the 66th and 67th amino acid of GLUT4. A PCR fragment of EGFP was inserted at the end of GLUT4. The final clone of Myc-GLUT4-EGFP was excised as EcoRI and XbaI and inserted into the modified pGreen Lantern Vector.

**Cell Culture and Electroporation of 3T3L1 Adipocytes**—CHO-T cells were grown in F-12 medium supplemented with 10% fetal bovine serum, 50 μg/ml streptomycin, and 50 units/ml penicillin. 3T3-L1 fibroblasts were cultured and differentiated into adipocytes as described previously (18). All GFP- or HA-tagged N-WASP and Myc-GLUT4-EGFP constructs were transfected into 3T3-L1 adipocytes by electroporation as described previously (13). Immunofluorescence microscopy—To visualize actin and endogenous N-WASP or Arp3, cells were fixed with 4% formaldehyde and permeabilized with a phosphate-buffered saline containing 1% fetal bovine serum and 0.5% Triton X-100 as described above. Cells were then incubated with primary rabbit anti-rat N-WASP or goat anti-human Arp3 antibody for 2 h at room temperature. After washing, cells were incubated with rhodamine-conjugated phallloidin together with FITC-labeled goat anti-rabbit IgG for N-WASP detection or with FITC-conjugated donkey anti-goat IgG for Arp3 detection for 30 min. After washing the coverslips were mounted in 90% glycerol containing 2.5% diazabicyclo[2.2.2]octane (DABCO). To examine Myc-GLUT4-EGFP translocation in adipocytes transfected with plasmid DNA, cells were washed, fixed, and immunostained with a set of antibodies using procedure described previously (36). Briefly, first, whole cell surface Myc-GLUT4-EGFP was detected with anti-myc monoclonal antibody and rhodamine B-labeled goat anti-mouse IgG. The cells were then permeabilized, and HA-tagged proteins were detected with polyclonal anti-HA antibody and Alexa-350-labeled goat anti-rabbit IgG.

**RESULTS**

**Insulin-mediated Actin Rearrangement Independent of PI 3-Kinase**—Although multiple studies have shown that insulin induces cell membrane ruffling, stress fiber breakdown, microspike, and cortical actin formation in different cell types (13, 18–24, 27, 28), the signaling pathways involved are not well characterized. Insulin action on membrane ruffling as detected by phallolidin staining of F-actin in the ruffles has been shown to be PI 3-kinase-dependent (8, 37–39). To test whether activation of PI 3-kinase by insulin is also required for dynamic changes in actin itself, we monitored insulin’s effects on F-actin formation using rhodamine-conjugated phallloidin in CHO-T cells and 3T3-L1 adipocytes. As shown in Fig. 1A, treatment of CHO-T cells with 100 nm insulin for 10 min induced the breakdown of stress fibers as shown in the upper panel images taken from optical planes at the bottom of cells in contact with the substratum. Insulin also induced striking membrane ruffling as shown in the lower panel images taken near the top of the cells. Treatment of the cells with wortmannin, a selective PI 3-kinase inhibitor, dramatically reduced membrane ruffling in almost all the CHO-T cells subsequently stimulated with insulin, although it did not significantly affect the formation of stress fibers or an apparent increase in cortical F-actin (Fig. 1B).

In contrast, 3T3-L1 adipocytes only showed small amounts of cortical F-actin are visualized at the bottom of the cells (Fig. 1B). However, insulin markedly increased cortical F-actin formation such that phallloidin-stained cell rims could easily be observed (Fig. 1B). Treatment of the cultured adipocytes with 100 nm wortmannin did not inhibit insulin’s effect on cortical F-actin formation (Fig. 1B), while at the same concentration it
Phospho-AktSer-473-specific antibody (Fig. 1) did block insulin-induced phosphorylation of Akt-1 detected by the presence or absence of 100 nM insulin. Cells were then washed with wortmannin (WM) for 20 min before further incubation for 10 min in the presence or absence of 100 nM insulin. Cells were then washed with ice-cold phosphate-buffered saline and fixed with 4% formaldehyde before detecting F-actin with rhodamine-conjugated phalloidin. Arrows point to membrane ruffles in A and adipocytes in B. C, the effect of wortmannin on insulin-induced serine 473 phosphorylation of Akt and total Akt protein levels were also detected with Western blotting as described under “Experimental Procedures.” Lane 1, control; lane 2, insulin 100 nM; lane 3, wortmannin (100 nM) and insulin (100 nM).

Insulin Causes N-WASP and Arp3 Co-localization with Actin Filaments Independent of PI 3-Kinase—The N-WASP-Arp2/3 pathway has been suggested to mediate actin polymerization in various types of cells (29–32, 34, 40, 41). To examine whether insulin acts through this signaling pathway, polyclonal antibodies against N-WASP and Arp3 were used to detect the localization of these endogenous proteins in 3T3-L1 adipocytes 7 days after initiation of differentiation. Fig. 2A shows that N-WASP expression is high in the nucleus and diffuse in the cytoplasm (Fig. 2A, panels d–f), whereas punctuate staining of Arp3 both in the cytoplasm and nucleus is evident (Fig. 2A, panels j–l). In response to insulin stimulation, phalloidin-stained F-actin filaments are readily seen in the cortex area of the cells and both N-WASP and Arp3 are co-localized with the newly formed cortical F-actin. However, inhibition of PI 3-kinase activity with wortmannin failed to affect either cortical F-actin formation or its co-localization with N-WASP and Arp3 in the cultured adipocytes, indicating these effects of insulin are PI 3-kinase independent.

Dominant Negative N-WASP-ΔWA Inhibits Insulin-mediated Cortical F-Actin Formation—To test whether N-WASP is involved in insulin-induced cortical F-actin formation, 3T3-L1 adipocytes were transfected with cDNA constructs encoding GFP-tagged N-WASP, ΔWH1 (WH1 domain-deleted) N-WASP and ΔWA (VCA domain-deleted) N-WASP (Fig. 3A), and insulin action on actin rearrangements examined. As shown in Fig. 3B, insulin induced striking cortical F-actin formation in adipocytes expressing either GFP alone, GFP-N-WASP, or GFP-N-WASP-ΔWH1, whereas cells expressing the GFP-N-WASP-ΔWA mutant significantly inhibited insulin-induced cortical F-actin formation. Among the cells expressing this latter construct, more than 75% of them showed spot-like aggregated actin filaments in the cytosol and around the nucleus (Fig. 3B, panel e), and only about 25% cells showed cortical F-actin in response to insulin (Fig. 3C). About 40% of the unstimulated adipocytes expressing the GFP-N-WASP-ΔWH1 mutant showed an increase in the formation of cortical F-actin (Fig. 3C), whereas the GFP-N-WASP-ΔWA mutant induced aggregated F-actin formation in the unstimulated cells (data not show). In addition, expression of GFP-N-WASP-ΔWH1 induced formation of cortical F-actin or microspike-like actin, and the loss of stress fibers in CHO-T cells, whereas the GFP-N-WASP-ΔWA significantly inhibited stress fiber breakdown induced by insulin in CHO-T cells (data not shown).

Further experiments were carried out to determine whether the dominant negative mutant N-WASP-ΔWA acts to inhibit insulin-stimulated F-actin formation by actually inhibiting plasma membrane recruitment of the endogenous N-WASP protein in 3T3-L1 adipocytes. Cells expressing GFP-N-WASP-ΔWA were stimulated with insulin for 10 min before immunostaining with rabbit polyclonal antibody against full-length rat N-WASP protein. GFP-N-WASP-ΔWA was predominantly localized in the nucleus, but some GFP fusion protein could be observed near the adipocyte plasma membrane in the basal state (Fig. 3D, panel m). Insulin treatment of the cells significantly increased both the GFP fusion protein (Fig. 3D, panel n) and immunoreactive N-WASP protein (Fig. 3D, panel l) at the plasma membrane. Since the antibody used in this study recognizes both endogenous full-length N-WASP and the expressed GFP-N-WASP-ΔWA, it is not possible to determine the extent to which each of these proteins contributes to the plasma membrane signal observed. However, as shown in both panels n and l of Fig. 3D, the GFP signal almost totally overlaps with the anti-N-WASP signal in the plasma membrane, suggesting the GFP-N-WASP-ΔWA is actually recruited to the plasma membrane where it can compete with endogenous N-WASP.

Dominant Negative TC10 (T31N) Blocks Insulin Signaling to N-WASP and Cortical F-actin—Multiple studies have shown that N-WASP interacts with the small GTP-binding protein Cdc42, leading to activation of the Arp2/3 complex and rapid actin polymerization (29–32, 34, 40, 41). N-WASP also interacts with TC10, a small GTPase closely related to Cdc42, in a yeast two-hybrid system assay (42). Insulin activates TC10 and expression of the dominant negative TC10 (T31N) mutant, but not the dominant negative mutants of Cdc42 (T17N) and Rac1 (T17N), inhibits insulin-induced GLUT4 translocation in 3T3-L1 adipocytes (17). In this study, we compared the effects of inhibitory mutants Cdc42 (T17N) and TC10 (T31N) on N-WASP recruitment to the cell periphery as well as insulin-induced actin rearrangement in 3T3-L1 adipocytes. Insulin markedly stimulated cortical localization of endogenous N-WASP (Fig. 4A, panel b, and 4B) and cortical F-actin formation (Fig. 4A, panel j, and 4C) in the cells expressing GFP alone.
Adipocytes expressing GFP-tagged cdc42 (T17N) exhibited the normal insulin effects to cause cortical localization of endogenous N-WASP (Fig. 4A, panel c, and 4B) and actin rearrangement (Fig. 4A, panel k, and 4C). However, insulin-induced accumulation of N-WASP in cortical area was also significantly reduced in TC10 (T31N) expressing cells (Fig. 4A, panel d, and 4B). Furthermore, insulin failed to induce cortical F-actin formation in at least 55% of the cells expressing GFP-tagged TC10.
It is interesting to note that those cells also displayed aggregated actin spots, similar to the cells expressing GFP-H9004WA-N-WASP mutant described above.

**Dominant Negative N-WASP-H9004WA Inhibits Insulin-induced GLUT4 Translocation**—To quantify GLUT4 translocation from intracellular compartments to the plasma membrane, we constructed the Myc-GLUT4-EGFP cDNA plasmid encoding GLUT4 tagged with a Myc epitope in its exofacial loop and with EGFP at its COOH terminus (Fig. 5A). The Myc epitope can be detected when on the cell surface by probing unpermeabilized cells with the combination of monoclonal anti-Myc antibody and rhodamine B-labeled anti-mouse IgG. Thus, GLUT4 translocation can be quantified by the ratio between intensities of rhodamine B on the cell surface and total EGFP in the cells expressing Myc-GLUT4-EGFP (Fig. 5, B and C). In 3T3-L1 adipocytes, insulin induced a rapid translocation of Myc-GLUT4-EGFP to the cell surface within 10 min, and its effect reached a maximal level within 45 min (data not shown). To examine the effects of N-WASP and its mutants on insulin-induced GLUT4 translocation, 3T3-L1 adipocytes were co-transfected with DNA constructs encoding Myc-GLUT4-EGFP and HA-tagged N-WASP or its mutants, followed by serum starvation and then insulin stimulation. As shown in Fig. 5, D and E, insulin increased cell surface Myc-GLUT4-EGFP by about 10-fold in control adipocytes expressing HA epitope alone. Expression of HA-tagged N-WASP and N-WASP-DWH1 did not alter insulin’s effects on Myc-GLUT4-EGFP translocation as compared with the cells expressing HA epitope alone in adipocytes. However, about 70–80% of adipocytes expressing HA-tagged N-WASP-DWA showed a reduced response or no response to insulin with respect to cell surface Myc-GLUT4-EGFP (Fig. 5E). Quantification of the average ratio of cell surface rhodamine B signal to total EGFP signal revealed a decrease of about 62% in insulin-stimulated adipocytes expressing HA-tagged N-WASP-DWA as compared with cells expressing HA epitope alone (Fig. 5D).

**DISCUSSION**

A key finding of the present studies is the remarkable contrast between insulin-mediated membrane ruffling versus cortical F-actin formation in their sensitivities to the PI 3-kinase inhibitor wortmannin (Fig. 1). The effect of insulin to cause membrane ruffling requires PI 3-kinase, consistent with many previously reported experiments using either wortmannin, blocking antibodies or dominant negative forms of PI 3-kinase (8, 37–39). Surprisingly, we find the effect of insulin on cortical F-actin formation in 3T3-L1 adipocytes to be insensitive to wortmannin under the same conditions (Fig. 1). This distinction of the membrane ruffling phenomenon compared with modulation of actin dynamics by insulin has apparently been missed in previous studies because the exaggerated F-actin bundles easily observed in membrane ruffles of fibroblasts are indeed inhibited along with the ruffles by wortmannin. In these cells, cortical F-actin is somewhat difficult to detect in the absence of ruffles, although at certain optical planes it can be observed (e.g. Fig. 1A, optical plane at the top of CHO-T cells in the presence of wortmannin). However, insulin-sensitive 3T3-L1 adipocytes the cortical F-actin formed in response to the hormone is readily observed whether or not PI 3-kinase is blocked (Figs. 1B and 2). We also note that this effect of insulin
to stimulate cortical F-actin polymerization is best observed when adipocytes are plated sparsely.

The potential impact of the above findings is magnified by the convergence of recent work (17) implicating a PI 3-kinase-independent pathway leading to TC10 is required in the control of glucose transport by insulin, combined with studies (13, 18–24) showing a requirement for intact F-actin in this process. Taken together, the data we obtained in Fig. 1 and these recent findings related to GLUT4 regulation led us to test whether indeed cortical F-actin polymerization may represent the target of this PI 3-kinase-independent insulin signaling. The results presented here strongly support this hypothesis. Insulin stimulates the movements of the TC10 target N-WASP and Arp3 to the cell periphery, even when PI 3-kinase is inhibited (Fig. 2). Furthermore, inhibitory forms of TC10 and N-WASP inhibit both insulin-mediated F-actin formation and GLUT4 translocation (Figs. 3–5 and Ref. 17). The specificity of the effect of TC10 (T31N) to inhibit insulin action on F-actin and GLUT4 recycling under conditions where dominant negative cdc42 (T17N) has no effect is striking, since both GTPases can interact with N-WASP in vitro (29–32, 34, 40–42). This specificity may derive from the recent report that TC10 expression is increased in differentiated 3T3-L1 adipocytes (43). In addition, this may be also related to the distinct localizations of these proteins whereby TC10 concentrates at the plasma membrane while Cdc42 is predominantly perinuclear (44). That N-WASP may be the physiological target of TC10 in 3T3-L1 adipocytes in a PI 3-kinase-independent signaling pathway is consistent with data in Fig. 4 showing inhibitory TC10 (T31N) blocks N-WASP movements triggered by insulin.

The present findings are consistent with recent studies that document a requirement of intact F-actin for optimal GLUT4 translocation in response to insulin in both primary (23) and cultured adipocytes (13, 18, 19, 21, 24). Interestingly, previous findings (18, 24) indicated the inhibition of insulin-stimulated GLUT4 recycling by actin depolymerization reagents such as latrunculin B is about 50%, similar to our present findings with dominant negative N-WASP (Fig. 5). This partial inhibition suggests that actin rearrangements facilitate GLUT4 movements and optimize efficiency of the translocation process. How might actin filaments near the plasma membrane promote GLUT4-containing membrane recycling? Two possibilities are suggested by studies with other systems involving secretory vesicle or granule movements in response to stimuli. One model invokes the requirement for localized active actin break-down and remodeling so that incoming membrane vesicles can reach the plasma membrane through the thick barrier of filaments in this region (45, 46). A second model suggests that unconventional myosins act to move membrane vesicles along actin filaments to sites of membrane fusion (47). These models are not mutually exclusive. Future work will be necessary to determine whether these or other mechanisms are involved in linking the PI 3-kinase-independent actin regulation described here to movements of vesicles that transport GLUT4 to the plasma membrane.

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REFERENCES

1. Czech, M. P. & Corvera, S. (1999) J. Biol. Chem. 274, 1865–1868
2. Pessin, J. E. & Saltiel, A. R. (2000) J. Clin. Invest. 106, 165–169
3. Shulman, G. I. (2000) J. Clin. Invest. 106, 171–176
4. Virkamaki, A., Ueki, K. & Kahn, C. R. (1999) J. Clin. Invest. 103, 931–943
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5. White, M. F. (1998) Mol. Cell. Biochem. 182, 3–11
6. Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J. & Kahn, C. R. (1994) Mol. Cell. Biol. 14, 4902–4911
7. Sharma, P. M., Egawa, K., Huang, Y., Martin, J. L., Huvar, J., Boss, G. R. & Olefsky, J. M. (1996) J. Biol. Chem. 271, 18528–18537
8. Martin, S. S., Haruta, T., Morris, A. J., Klippe, A., Williams, L. T. & Olefsky, J. M. (1996) J. Biol. Chem. 271, 17665–17668
9. Jiang, T., Sweeney, G., Rudolf, M. T., Klip, A., Traynor-Kaplan, A. & Tsien, R. Y. (1998) J. Biol. Chem. 273, 11017–11024
10. Isakoff, S. J., Taha, C., Rose, E., Marcusohn, J., Klip, A. & Skolnik, E. Y. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10247–10251
11. Guilherme, A. & Czech, M. P. (1998) J. Biol. Chem. 273, 33119–33122
12. Kannaki, M., Watson, R. T., Artemyev, N. O. & Pessin, J. E. (2000) J. Biol. Chem. 275, 7167–7175
13. Buse, A., Cherniack, A. D., Langille, S. E., Nicolör, S. M. C., Buxton, J. M., Park, J. G., Wang, L., Blenis, J. & Kahn, C. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1197–1201
14. Lawrence, J. T. R. & Birnbaum, M. J. (2001) Mol. Cell. Biol. 21, 2993–3002
15. Kennedy, J. W., Hirshman, M. F., Gervino, E. V., Ocel, J. V., Forse, R. A., Hoening, S. J., Aronson, D., Goodyear, L. J. & Horton, E. S. (1999) Diabetes 48, 1192–1197
16. Baumann, C. A., Ribo, V., Kannaki, M., Thurmond, D. C., Mora, S., Shigematsu, S., Bickel, P. E., Pessin, J. E. & Saltiel, A. R. (2000) Nature 407, 202–207
17. Chiang, S. H., Baumann, C. A., Kannaki, M., Thurmond, D. C., Watson, R. T., Neudauer, C. L., Macara, I. G., Pessin, J. E. & Saltiel, A. R. (2001) Nature 410, 944–948
18. Emoto, M., Langille, S. E. & Czech, M. P. (2001) J. Biol. Chem. 276, 10677–10682
19. Guilherme, A., Emoto, M., Buxton, J. M., Buse, S., Sabini, R., Theurkauf, W. E., Leszyk, J. & Czech, M. P. (2000) J. Biol. Chem. 275, 38151–38159
20. Tsakiridis, T., Vranic, M. & Klip, A. (1994) J. Biol. Chem. 269, 17162–17167
21. Wang, Q., Somwar, R., Bilan, P. J., Liu, Z., Jin, J., Chawla, A., Corvera, S. & Czech, M. P. (1999) J. Biol. Chem. 274, 27089–27101
22. Asahi, Y., Hayashi, H., Wang, L. & Ebina, Y. (1999) J. Biol. Chem. 274, 19196–19201
23. Park, J. G., Chawla, A. & Czech, M. P. (2001) J. Biol. Chem. 276, 19196–19201
24. Patki, V., Buxton, J., Chawla, A., Lifshitz, L., Fogarty, K., Carrington, W., Tuft, R. & Corvera, S. (2001) Mol. Biol. Cell 12, 129–141
25. Fletcher, L. M., Welsh, G. I., Oatey, P. B. & Tavare, J. M. (2000) Biochem. J. 352, Pt 2, 267–276
26. Olsen, A. L., Trumbly, A. R. & Gibson, G. V. (2001) J. Biol. Chem. 276, 10706–10714
27. Tsakiridis, T., Isakoff, S. J., Rose, E., Symons, M. & Skolnik, E. Y. (1995) Curr. Biol. 5, 1296–1302
28. Nakashima, N., Rose, D. W., Xiao, S., Egawa, K., Martin, S. H., Haruta, T., Saltiel, A. R. & Olefsky, J. M. (1999) J. Biol. Chem. 274, 3001–3008
29. Higgs, H. N. & Pollard, T. D. (2001) Annu. Rev. Biochem. 70, 649–676
30. Takenawa, T. & Miki, H. (2001) J. Cell Sci. 114, 1801–1809
31. Rohatgi, R., Ma, L., Miki, H., Lopes, M., Kirchhausen, T., Takenawa, T. & Kirschner, M. W. (1999) Cell 97, 221–231
32. Rohatgi, R., Ho, H. Y. & Kirschner, M. W. (2000) J. Cell Biol. 150, 1299–1310
33. Langille, S. E., Patki, V., Klurand, J. K., Buxton, J. M., Holik, J. J., Chawla, A., Corvera, S. & Czech, M. P. (1999) J. Biol. Chem. 274, 27089–27101
34. Moreau, V., Frischknecht, F., Beckmann, I., Vincentelli, R., Rabut, G., Stewart, D. & Way, M. (2000) Nat. Cell Biol. 2, 441–448
35. Murphy, G. A., Soloki, P. A., Jillian, S. A., Perez de la Osa, P., D'Eustachios, P., Der, C. J. & Rush, M. G. (1999) Oncogene 18, 3931–3945
36. Czech, M. P., Chawla, A., Woon, C. W., Buxton, J., Armoni, M., Tang, W., Joly, M. & Corvera, S. (1993) J. Cell Biol. 123, 127–135
37. Khayat, Z. A., Long, A., Ebert, M. & Klip, A. (2000) J. Cell Sci. 113, 279–290
38. Wang, Q., Somwar, R., Bilan, P. J., Liu, Z., Jin, J., Wodgett, J. R. & Klip, A. (1999) Mol. Cell. Biol. 19, 4008–4018
39. Siddhanta, U., McIvor, J., Shah, A., Zhang, Y. & Backer, J. M. (1998) J. Cell Biol. 143, 1647–1659
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