Counter-regulation by insulin and isoprenaline of a prominent fat-associated phosphoprotein doublet in rat adipocytes

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1. In the adipocyte, phosphorylation/dephosphorylation of regulatory proteins is a common mechanism of metabolic regulation. We have observed a very prominent phosphoprotein doublet of 61 kDa and 63 kDa in rat adipocytes that is markedly responsive to hormones. The 63 kDa band was the predominant phosphoprotein in the cell in response to 0.1 μ M-isoprenaline, whereas the 61 kDa band was nearly absent. Insulin alone did not alter ^{32} P incorporation into the doublet, but partially counteracted the effects of isoprenaline, decreasing label in the 63 kDa band by as much as 50% and resulting in the reappearance of the 61 kDa band. 2. Subcellular fractionation demonstrated that both phosphoprotein bands were fat-associated. Neither insulin nor isoprenaline altered this localization. Peptide maps (one-dimensional) of the 61/63 kDa bands demonstrated close sequence similarity. Amino acid analysis revealed the presence of phosphoserine and phosphothreonine. The latter was more prominent in the 61 kDa band. Isoprenaline caused an absolute increase in both phosphoamino acids. 3. Permeabilization of ^{32} P-labelled isoprenaline-treated cells with digitonin initiated rapid dephosphorylation of the 63 kDa band, with reappearance of the 61 kDa band. Insulin increased the rate of dephosphorylation by 2–3-fold when present with isoprenaline before permeabilization. 4. In permeabilized adipocytes, cyclic AMP (1 μ M–1 mM) increased phosphorylation of the 61/63 kDa doublet by 4–10-fold in the presence of [γ - ^{32} P]ATP, but insulin had no effect. 5. We conclude that this prominent phosphoprotein, migrating as a 61/63 kDa doublet, is coupled to the cyclic AMP-dependent protein kinase and is associated with an insulin-stimulated phosphoprotein phosphatase activity. This fat-associated phosphoprotein, which is under counter-regulatory hormonal control, may play a role in hormone-dependent lipid metabolism.

INTRODUCTION

Serine/threonine phosphorylation plays a central role in the regulation of many insulin-dependent enzymes in adipocyte metabolism. In the mid-1970s, Benjamin & Singer [1] and Avruch *et al.* [2] demonstrated the diverse effects of insulin and catecholamines on protein phosphorylation in rat adipocytes. These groups and others have reported a very prominent adrenaline-dependent phosphorylated protein of 60–70 kDa. Surprisingly, this prominent 60–70 kDa phosphoprotein has not been identified and only now is being investigated by us [3] and others [4].

Cyclic AMP-dependent phosphorylation of proteins such as the enzymes glycogen synthase [5,6] and hormone-sensitive lipase [7] has been extensively characterized. Insulin inhibits these cyclic AMP-dependent phosphorylations and alters enzyme activity, but the mechanism by which insulin directly mediates these effects is unclear. Some evidence indicates that insulin inhibits the cyclic AMP kinase [8], whereas others suggest that increased phosphatase activity may be responsible [9]. Other potential sites are adenylate cyclase [10,11] and the low- K_m phosphodiesterase [12,13].

Here we demonstrate that the electrophoretic mobility of a 61/63 kDa isoprenaline-sensitive phosphoprotein doublet, which is probably the 60–70 kDa phosphoprotein reported by Benjamin & Singer [1] and Avruch *et al.* [2], is under counter-regulatory hormonal control and appears to be converted between multiple phosphorylation states. Insulin decreases isoprenaline-dependent ^{32} P incorporation into this protein and increases its electrophoretic mobility. We present evidence that this protein doublet is located on the fat droplet, is coupled to the cyclic AMP-

dependent protein kinase and is associated with an insulin-sensitive phosphatase.

MATERIALS AND METHODS

Male Sprague–Dawley rats, weighing 125–150 g, were purchased from the Charles River Breeding Laboratory (Wilmington, MA, U.S.A.). Collagenase (Type I) was from Worthington (Freehold, NJ, U.S.A.), and [^{32} P] P_i was from New England Nuclear (Boston, MA, U.S.A.). Insulin was generously provided by Lilly Laboratories (Indianapolis, IN, U.S.A.). BSA (Cohn Fraction V), 8-bromo cyclic AMP, and N^6 -monobutyl cyclic AMP were from Sigma (St. Louis, MO, U.S.A.). Digitonin was from ICN Biochemicals (Cleveland, OH, U.S.A.), and cellulose t.l.c. plates were from Analtech (Newark, DE, U.S.A.). [γ - ^{32} P]ATP was synthesized using the enzymic procedure of Walseth & Johnson [14].

Phosphorylation reactions

Rat adipocytes were isolated by collagenase digestion of epididymal fat pads by the method of Rodbell [15]. Adipocytes were suspended at 2×10^6 cells/ml in carrier-phosphate-free Krebs–Ringer/Hepes (50 mM) containing [^{32} P] P_i (60 μ Ci/ml), 11.1 mM-glucose and 30 mg of BSA/ml, and pipetted (0.5 ml) into Microfuge tubes. After 2 h at 37 °C, insulin, isoprenaline or other effectors were added for a subsequent 5 min incubation. Cells were rapidly recovered by spinning through silicone oil, quick-frozen in ethanol/solid CO_2 , solubilized in cold stopping solution [62 mM-Tris (pH 6.9), 2% SDS, 10% glycerol, 5% β -mercaptoethanol, 100 mM-dithiothreitol, 2 mM-vanadate, 1 mM-*p*-nitrophenol phosphate, 1 mM-phenylmethanesulphonyl

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fluoride, 100 000 units of aprotinin and 1 mg of benzamidine/ml] and subsequently placed in a boiling-water bath for 5 min. Samples were subjected to SDS/PAGE on 7.5% or 5–15% gels and phosphoproteins were localized by autoradiography. Quantification of labelling with ^{32}P was performed by excising the bands of interest and measuring the radioactivity by Čerenkov counting.

Dephosphorylation reactions

These were initiated after the hormone treatments by removing the labelling medium with a syringe and replacing it with permeabilization medium containing 20 mM-Tris (pH 7.4), 125 mM-KCl, 5 mM-NaCl, 5 mM-MgCl₂ (or 10 mM-EDTA) and 20 μg of digitonin/ml. Reactions were stopped at various time points and samples were processed as above.

Phosphorylation reactions in permeabilized adipocytes

Labelling of permeabilized adipocytes was performed as previously described [16] with minor variations. In brief, rat adipocyte suspensions (2×10^6 cells/ml) were permeabilized for 30 min in medium containing 20 mM-Tris (pH 7.4), 125 mM-KCl, 5 mM-NaCl, 5 mM-MgCl₂, 1% (w/v) BSA, 11 mM-glucose and 20 μg of digitonin/ml. Labelling medium was then added for 2 min; this contained Tris medium (without BSA and digitonin) plus 150 μM -[^{32}P]ATP (1 $\mu\text{Ci}/\text{nmol}$). Insulin and cyclic AMP were added where indicated. Reactions were stopped and samples were processed as above.

Phosphoamino acid analysis

Phosphoproteins were electroblotted from SDS/PAGE gels to silicon-coated glass fibre membranes (Biometra Glassybond; Immunetics, Cambridge, MA, U.S.A.) [17] and localized by autoradiography. Bands were subjected to direct acid hydrolysis (6 M-HCl; 2 h at 110 °C); phosphoamino acids were purified on Dowex 50-X8 (H⁺ form) [18], and separated by t.l.c. at pH 3.5.

One-dimensional peptide mapping techniques

Phosphoprotein bands from dried (but not acid-fixed) gels were excised, rehydrated and applied to the wells of a 15% PAGE gel in the presence of SDS according to the procedure of Cleveland *et al.* [19]. *Staphylococcus aureus* V8 proteinase or chymotrypsin was overlaid on the gel pieces and electrophoresed into the stacking gel. After a 15 min incubation the samples were electrophoretically separated, the gels were fixed and dried, and the resulting phosphopeptides were revealed by autoradiography.

RESULTS

An examination of total cellular phosphorylated proteins in rat adipocytes revealed two very prominent phosphoprotein bands of comparable intensity at 61 kDa and 63 kDa. Maximum resolution of the two bands required only short autoradiographic exposures, conditions which were insufficient to detect most other phosphoproteins. Isoprenaline at 0.1 μM increased incorporation of ^{32}P label into the 63 kDa band by 4–5-fold and caused the near-complete loss of the 61 kDa band (Fig. 1a). Insulin alone at 1 nM had no appreciable effect on the phosphoprotein doublet. Insulin partially counteracted the effect of isoprenaline, however (Fig. 1b). The intensity of the 63 kDa band was decreased in the presence of insulin and isoprenaline together compared with isoprenaline alone, and the 61 kDa band reappeared, although the doublet could not always be resolved under the combined effects of both hormones.

Dose-dependent phosphorylation of the 61/63 kDa doublet by two cyclic AMP analogues, *N*⁶-monobutyl cyclic AMP and 8-bromo cyclic AMP, demonstrated increased incorporation of

label into both the 61 kDa and the 63 kDa bands at low agonist concentrations (Fig. 2). At the highest agonist concentrations tested, the 61 kDa band displayed decreased electrophoretic mobility (especially apparent with 8-bromo cyclic AMP), and incorporation of label into the 61 kDa and 63 kDa bands was twice that of basal. Insulin at 1 nM counteracted the effect of 8-bromo cyclic AMP by approx. 50% at all analogue concentrations (Fig. 2d). In contrast, *N*⁶-monobutyl cyclic AMP resisted inhibition by insulin (Fig. 2c).

Reciprocal appearance of the 61 kDa and 63 kDa bands, their decreased electrophoretic resolution in the presence of both insulin and isoprenaline, and the altered electrophoretic mobility of the 61 kDa band in response to cyclic AMP analogues, suggested that the bands represent one polypeptide which is multiply phosphorylated and exhibits different mobility in response to its multiple phosphorylated states or levels of phosphorylation.

One-dimensional peptide maps of the 61 kDa and 63 kDa bands, prepared according to the method of Cleveland *et al.* [19] from adipocytes without hormone treatment, provided strong evidence that the 61 kDa and 63 kDa phosphoprotein bands represented one polypeptide. Both the chymotrypsin (Fig. 3a) and V8 proteinase (Fig. 3b) maps demonstrated close peptide similarity between the two species. In the chymotrypsin maps all phosphopeptides of more than 29 kDa derived from the 61 kDa band were matched to corresponding peptides from the 63 kDa band. In the V8 proteinase map, close similarity was again apparent between the phosphopeptides from the 61 kDa and 63 kDa bands. Only a few distinct differences were apparent.

Fig. 1. Counter-regulatory effect of insulin on isoprenaline-dependent phosphorylation of the 61/63 kDa doublet

Rat adipocyte suspensions (1×10^6 cells/0.5 ml) were exposed to isoprenaline (Iso, 0.1 μM) and insulin, as indicated, for 5 min after a 2 h incubation with carrier-free [^{32}P]P_i. Cells were rapidly recovered by centrifugation and the phosphoproteins were separated by SDS/PAGE. Results were analysed by a 20 min autoradiographic exposure (a) and by excising the bands and measuring the radioactivity by Čerenkov counting (b). □, Isoprenaline + insulin; ■, insulin alone.

Fig. 2. Dose-dependent effects of cyclic AMP analogues on phosphorylation of the 61/63 kDa doublet

Rat adipocyte suspensions (1×10^6 cells/0.5 ml) were exposed to *N*⁶-monobutyl cyclic AMP (mb cAMP; a,c) or 8-bromo cyclic AMP (8-br cAMP; b,d) for 5 min after a 2 h incubation with carrier-free [³²P]P_i. Insulin (1 nM, ■) was included where indicated. Samples were processed as in Fig. 1. Results were analysed by a 30 min room temperature autoradiographic exposure (a,b) and by excising the bands and measuring the radioactivity by Čerenkov counting (c,d).

Fig. 3. One-dimensional peptide maps of the 61 kDa and 63 kDa bands

The 61 and 63 kDa bands, prepared as in Fig. 1 in the absence of hormones, were excised and subjected to Cleveland mapping [19] on a 15% gel in the presence of SDS, followed by autoradiographic visualization. Chymotrypsin concentrations were 0.5 μg (lanes 1 and 3) and 5 μg (lanes 2 and 4) per lane (a) and *Staph. aureus* V8 proteinase concentrations were 0 (lanes 1 and 4), 0.05 (lanes 2 and 5), and 0.5 μg (lanes 3 and 6) per lane (b). Note that some V8 proteinase contaminated the control lane for the 61 kDa band.

Fig. 4. Phosphoamino acid analysis of the 61 kDa and 63 kDa bands

Rat adipocyte suspensions (1×10^6 cells/0.5 ml) were exposed to isoprenaline (iso, 0.1 μM) and/or insulin (ins, 1.0 nM) as in Fig. 1. Phosphoproteins were separated by SDS/PAGE, electrotransferred to siliconized glass fibre membranes and subjected to direct acid hydrolysis as described in the Materials and methods section. Lanes 1-4, 63 kDa protein; lanes 5-8, 61 kDa protein.

Phosphoamino analysis of the 61 kDa and 63 kDa phosphoprotein bands from labelled intact cells, when excised from SDS/PAGE gels, revealed the presence of both phosphoserine and phosphothreonine, with no phosphotyrosine detected under these experimental conditions (Fig. 4). In the absence of hormonal treatment, phosphoserine was the more prominent phosphoamino acid in the 63 kDa band, whereas phosphothreonine appeared to be at least as abundant as phosphoserine in the 61 kDa band. Insulin had no apparent effect on the distribution of phosphoamino acids, whereas isoprenaline caused an absolute increase in both phosphoamino acids, with the expected shift of label from the 61 kDa band to the 63 kDa band.

Subcellular fractionation of labelled adipocytes revealed that the 61/63 kDa doublet was present almost exclusively in the fat cake (Fig. 5). Little was detected in the 140 000 g crude membrane pellet and none was detected in the 140 000 g supernatant. Hormonal treatment appeared not to affect the phosphoprotein distribution.

Fig. 5. Subcellular localization of the 61/63 kDa doublet

Rat adipocytes were labelled as in Fig. 1, isolated by centrifugation and homogenized in the presence of phosphatase and proteinase inhibitors. Phosphoproteins from the fat cake (1000 g), a crude membrane fraction (140000 g pellet) and a cytosolic fraction (140000 g supernatant) were recovered and separated by SDS/PAGE. Iso, isoprenaline.

Fig. 6. 'In situ' phosphorylation in permeabilized adipocytes

Rat adipocyte suspensions (1×10^6 cells/0.5 ml) were preincubated for 30 min in medium containing digitonin at the concentrations indicated. Labelling medium containing $150 \mu\text{M}$ - ^{32}P ATP ($1 \mu\text{Ci/nmol}$) and $10 \mu\text{M}$ -cyclic AMP was then added for 2 min. Cells were recovered and phosphoproteins were isolated as in Fig. 1.

Fig. 7. Effect of cyclic AMP and insulin on 61/63 kDa doublet phosphorylation in permeabilized adipocytes

Adipocyte suspensions were preincubated with $20 \mu\text{g}$ of digitonin/ml for 30 min as in Fig. 5. Insulin, cyclic AMP and ^{32}P ATP were then added as indicated for 2 min. Phosphoproteins were isolated and revealed by autoradiography.

Fig. 8. Initiation of dephosphorylation by permeabilization

Rat adipocyte suspensions (1×10^6 cells/0.5 ml), prelabelled for 2 h with ^{32}P ATP, were exposed to isoprenaline ($0.1 \mu\text{M}$) alone (a) or with insulin (1.0 nM) for 5 min, as in Fig. 1. Permeabilization was then initiated with a medium change to one containing digitonin ($20 \mu\text{g/ml}$) and Mg^{2+} (5 mM). Incubations were terminated at the times indicated and samples were processed as in Fig. 1. This experiment was performed three times with similar results.

Not only did the 61/63 kDa doublet represent the predominant phosphoprotein bands in labelled intact adipocytes, but these bands were also most dominant when adipocytes were permeabilized with digitonin ($20 \mu\text{g/ml}$) for 30 min and cellular proteins were subsequently labelled with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ in the presence of cyclic AMP. No significant labelling was detected at 0 or $5 \mu\text{g}$ of digitonin/ml, but maximum labelling was observed at concentrations of $10 \mu\text{g/ml}$ and above (Fig. 6). The 61 kDa band was the more prominently labelled band at $1 \mu\text{M}$ -cyclic AMP, and both the 61 kDa and 63 kDa bands were extensively labelled at higher cyclic AMP concentrations, with maximum effects at $100 \mu\text{M}$ (Fig. 7). Insulin had no effect on this phosphorylation when added to the permeabilized adipocytes. An additional cyclic AMP-dependent phosphoprotein of approx. 46 kDa was also labelled under these conditions. This protein was also fat-associated. With extended autoradiographic exposure, a total of 15–18 phosphoproteins was observable. Three additional proteins were labelled in a cyclic AMP-dependent manner, 80, 115 and 175 kDa, with the latter two not always resolved.

Permeabilization was also used as a technique to investigate cellular dephosphorylation of the 61/63 kDa doublet. Intact adipocytes were labelled, as described above, for 2 h and then treated with hormones for 5 min. Permeabilization was then rapidly initiated by replacing the suspension medium with one containing digitonin. In isoprenaline-treated adipocytes, ^{32}P label in the 63 kDa band decreased markedly during the subsequent 6 min interval and the 61 kDa band reappeared. Insulin, when added with isoprenaline before permeabilization, increased the apparent rate of this dephosphorylation reaction by 2–3-fold (Fig. 8). Insulin did not markedly alter the final intensity of the 61 kDa band. The 61 kDa band was never completely dephosphorylated, even with extended incubations, regardless of the hormonal conditions. Dephosphorylation of the 61/63 kDa band was temperature-dependent, with near-complete inhibition at 12°C . NaF (50 mM) inhibited dephosphorylation by 16% ($n = 3$) when measured at 1 min. Maximum activity of the dephosphorylation reaction required magnesium. Both the rate and extent of the reaction were inhibited by EDTA (results not shown).

Other phosphoprotein bands were variably affected by permeabilization. No bands below 75 kDa (excluding the 61/63 kDa doublet) were appreciably decreased. Several bands larger than 75 kDa decreased in parallel, all losing an equal percentage of their initial label during the course of the

dephosphorylation experiment (the range observed in multiple experiments was 10–50 %). The 80 kDa cyclic AMP-dependent band was also very rapidly lost, being essentially absent beyond the first dephosphorylation time point. Whether these decreases represent enzyme-mediated dephosphorylation or loss of freely diffusible cytosolic proteins from the permeabilized cells remain to be determined.

DISCUSSION

The 61/63 kDa doublet described in this report is the dominant phosphoprotein in rat adipocytes when these cells are exposed to isoprenaline or other agents which raise intracellular cyclic AMP. The protein appears to have multiple phosphorylation sites (on both serine and threonine) which are under counter-regulatory control by cyclic AMP and insulin. Its changing mobility on SDS/PAGE is apparently a result of charge differences between the various phosphorylation states or levels of total phosphorylation. Despite these notable characteristics, this protein has not been previously characterized nor has its function yet been determined.

The 61/63 kDa phosphoprotein was associated with the fat droplet under all the hormonal conditions tested. The permeabilization studies indicate that the protein is in close proximity to the cyclic AMP-dependent protein kinase and is accessible to a phosphoprotein phosphatase. The relationship with the cyclic AMP kinase reaction is of particular interest, since the phosphorylation reaction remained functional for 30 min after permeabilization of the adipocytes. The permeabilization conditions are sufficient to permit loss of both low-molecular-mass compounds and freely diffusible proteins from the cell [20]. Resistance of the kinase reaction to these conditions suggests that the kinase and the 61/63 kDa doublet are tightly associated and not easily uncoupled. A similar relationship may also be true for several other cyclic AMP-dependent phosphoproteins which were observed, including the 46, 80, 115 and 175 kDa bands.

Permeabilization also permitted an examination of dephosphorylation of the 61/63 kDa doublet. The dephosphorylation reaction involved primarily a conversion of the doublet from the 63 kDa form to the 61 kDa form, with a net loss of label. This represented reversal of the isoprenaline-dependent phosphorylation pattern and provides additional support for the interpretation that the doublet represents multiple phosphorylation states of one protein. Preservation of the dephosphorylation reaction after permeabilization also suggests that a phosphatase(s) may be spatially associated with the phosphoprotein, and, as such, may also be associated with the lipid droplet structure. Additional studies will be necessary to investigate this possibility.

The mechanism by which insulin antagonizes the effects of cyclic AMP in adipocytes is unclear. It has been proposed by Londos *et al.* [9] that insulin antagonizes the lipolytic effects of low cyclic AMP concentrations (protein kinase A activity ratios below 0.25) by decreasing cyclic AMP concentrations. Activation of the low- K_m phosphodiesterase might be the mechanism by which insulin mediates this effect. At intermediate cyclic AMP concentrations (protein kinase A ratios below 0.6), a cyclic AMP-independent activation of a phosphatase by insulin is proposed to be the mechanism. Finally, at very high cyclic AMP concentrations (protein kinase A activity ratios > 0.6) the effect of insulin is lost entirely.

Our demonstration that insulin increased the rate of the dephosphorylation reaction suggests activation of a phosphoprotein phosphatase by the hormone, and may support the model of Londos *et al.* [9]. It is not clear, however, whether

our results represent a cyclic AMP-independent activation of a phosphatase by insulin or reversal of a cyclic AMP-dependent inhibition of a phosphatase. Regardless, a cyclic AMP-independent activation of a phosphatase by insulin does not appear to be the major mechanism by which insulin counter-regulates isoprenaline-dependent phosphorylation of the 61/63 kDa doublet under our conditions. This conclusion is based on experiments with *N*⁶-monobutyl cyclic AMP, a poor substrate for the cyclic AMP phosphodiesterase, and 8-bromo cyclic AMP, a good phosphodiesterase substrate [21,22]. Both cyclic AMP analogues mimicked the effects of low isoprenaline concentrations on phosphorylation of the doublet. However, insulin counteracted the effects of 8-bromo cyclic AMP as with isoprenaline but had essentially no effect on monobutyl cyclic AMP-dependent phosphorylation. These results suggest the importance of insulin-dependent activation of cyclic AMP phosphodiesterase and modulation of intracellular cyclic AMP concentrations in mediating the effects of insulin on the 61/63 kDa phosphoprotein. If insulin-regulated phosphatase activity is a major mechanism for reversing the isoprenaline effect, it is more likely to be regulated indirectly through decreased cyclic AMP concentrations and removal of cyclic AMP-dependent inhibition of a phosphatase. These results do not exclude the possibility, however, that insulin does directly activate a phosphoprotein phosphatase independent of cyclic AMP.

An alternative interpretation for the insulin-dependent increase in the rate of dephosphorylation is that insulin altered the phosphoprotein in such a way that it became a better substrate for a constitutive phosphatase. That is, the effect of insulin was mediated not on the phosphatase but on the substrate. Since the protein appears to be multiply phosphorylated under counter-regulatory control by isoprenaline and insulin, it is possible that each phosphorylation site dephosphorylates at a unique rate. Although this possibility cannot be ruled out, we believe that this mechanism is less likely. When the effect of insulin on net isoprenaline-dependent phosphorylation of the 61/63 kDa doublet is examined in intact cells, as in Fig. 1 of this paper, the magnitude of the effect is variable. However, the magnitude of the insulin effect on net phosphorylation does not appear to affect the ability of insulin to increase the rate of dephosphorylation. In the experiment depicted in Fig. 8, the effect of insulin on isoprenaline-dependent phosphorylation in the intact cells was small, with negligible conversion from the 63 kDa phosphorylated state (see zero time phosphorylation $\pm$ insulin). Despite the lack of a notable effect of insulin on net phosphorylation, the effect of insulin on subsequent dephosphorylation was clear. Thus the phosphorylation state of the 61/63 kDa doublet does not appear to be critical for observing the effect of insulin on the rate of dephosphorylation. These results also suggest that insulin-dependent dephosphorylation is not the major determinant of the phosphorylation state of the protein under these conditions.

In summary, we have demonstrated that the dominant cyclic AMP-dependent phosphoprotein in the rat adipocyte has multiple phosphorylated forms or levels of phosphorylation which result in electrophoretic migration differences. Interconversion of the protein between these phosphorylated states is counter-regulated by insulin and cyclic AMP. This protein is fat-associated and is tightly associated with the cyclic AMP-dependent protein kinase. One mechanism by which insulin regulates the phosphorylation state of the protein appears to be through activation of the dephosphorylation reaction. Although the function of the 61/63 kDa protein is not yet known, its subcellular localization to the fat droplet and its association with hormonally regulated phosphorylation and dephosphorylation reactions suggest that it may play a role in lipid metabolism in the adipocyte.

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