RESEARCH COMMUNICATION

An insulin receptor peptide (1135–1156) stimulates guanosine 5'-[γ -thio]triphosphate binding to the 67 kDa G-protein associated with the insulin receptor

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Peptides representing two putative G-protein-binding motifs (GPBP1 and GPBP2) derived from insulin-receptor sequences were tested for their ability to stimulate guanosine 5'-[γ -thio]triphosphate (GTP[S]; 'GTP γ S') binding to a preparation containing the 41 and 67 kDa G-proteins that are associated with the insulin receptor [Jo, Cha, Davis and McDonald (1992) *Endocrinology* (Baltimore) 131, 2855–2861]. GPBP2 (residues 1135–1156) specifically stimulated GTP[S] binding, whereas GPBP1 (1319–1333) did not. Substitution of Arg-1152 with Gln in

GPBP2 corresponding to a mutation site in insulin-resistant patients [Cocozza, Porcellini, Riccardi, Monticelli, Condorelli, Ferrera, Pianese, Miele, Capaldo, Beguinot and Varrone (1992) *Diabetes* 41, 521–526] attenuated the stimulatory potency of GPBP2. Size-exclusion chromatography and studies with purified 67 kDa G-protein revealed that GPBP2 stimulated GTP[S] binding only to the 67 kDa G-protein. These studies provide evidence for a potential regulatory site for G-protein interaction with the insulin receptor in the tyrosine kinase domain.

INTRODUCTION

Insulin activates the β -subunit of the insulin receptor by binding to the α -subunit of the receptor. The activated insulin receptor becomes autophosphorylated [1] and triggers a cascade of phosphorylation/dephosphorylation events [2]. Target proteins that are phosphorylated by the activated receptor tyrosine kinase include insulin-receptor substrate-1 (IRS-1) [3], phosphatidylinositol-3-kinase [4], GTPase-activating protein [5], calmodulin [6] and fatty-acid-binding protein [7]. Although the phosphorylation cascade represents a significant mechanism for signal transduction in insulin action, the detailed molecular mechanisms that regulate the coupling of the insulin receptor kinase to target proteins remain elusive.

G-proteins are a family of signal transducers for a variety of receptor systems [8,9]. GTP [10–16], cholera-toxin-sensitive G-proteins [presumably stimulatory G-proteins (G_s)] [17,18], pertussis-toxin-sensitive G-proteins [presumably inhibitory G-protein (G_i)-like proteins] [19,20] and p21 ras G-protein [21] have been reported to mediate some of the actions of insulin. The effects of GTP or guanosine 5'-[γ -thio]triphosphate (GTP[S]) on insulin-receptor kinase activity appear to be cell-type-specific. GTP or GTP[S] inhibit insulin receptor kinase activities of plasma membranes [13] and wheat-germ-purified insulin receptor of rat adipocytes [12] and insulin-stimulated tyrosine phosphorylation of a 160 kDa protein in permeabilized L6 cells [11]. In contrast, the insulin-receptor kinase purified from cardiac myocytes is stimulated by GTP[S] [16]. Likewise, insulin-stimulated microtubule-associated protein-2 kinase and ribosomal S6 kinase activities of 3T3-L1 adipocytes can be stimulated by GTP[S] [14].

These results indicate that the insulin receptor may be coupled to different G-proteins in different cells. Such alternative coupling could explain why hexose transport of rat adipocytes [19] and BC3H1 cells [20] is sensitive to pertussis toxin, whereas in 3T3-L1 adipocytes [17] and cardiac myocytes [18] it is sensitive only to cholera toxin.

We have identified two G-proteins of 67 and 41 kDa that are associated with the insulin receptor [22]. Removal of these G-proteins from the insulin receptor results in elevated basal tyrosine kinase activity and concomitant loss of the insulin-sensitivity [22]. Srivastava and Singh [23] have also shown that a similar 66 kDa G-protein is coupled to the insulin receptor. In addition, a 68 kDa G-protein associated with the T-cell receptor [24] and a 74 kDa G-protein associated with the α_1 -adrenergic receptor [25] have been identified. These results suggest that there may be a novel class of 66–74 kDa G-proteins coupled to receptors that are not traditionally thought to be associated with G-proteins.

Several studies have identified G-protein-binding motifs either by mutating specific regions of receptors [26–28] or by using synthesized peptides based on the primary amino acid sequence of receptors [29–30]. Nishimoto and colleagues [29,30] have shown that a peptide sequence comprised of basic amino acids (B) in the N-terminus and BBXXB in the C-terminus (X is any amino acid) is the critical recognition motif for G-protein binding to β -adrenergic and insulin-like growth-factor-II (IGF-II) receptors (see Figure 1a). We have identified two motifs in the insulin-receptor β -subunit that fulfil these criteria. These motifs are shown in Figure 1 and are identified as IR_{GPBP1} and IR_{GPBP2} [(G-protein-binding peptides (GPBP) 1 and 2)]. The locations in

Abbreviations used: GTP[S], guanosine 5'-[γ -thio]triphosphate ('GTP γ S'); the amino acid sequence numbering system is that of Ullrich et al. [41]; GPBP1 and 2, G-protein-binding peptides 1 and 2; CP, a 22-mer control peptide that does not fulfil the criteria of G-protein-binding peptide; G_i , an inhibitory G-protein; G_s , a stimulatory G-protein; DTT, dithiothreitol; IRS-1, insulin-receptor substrate 1; IGF-II, insulin-like growth factor-II; ISFT, insulin-Sepharose Flow-through.

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Figure 1 G-protein binding motifs in the insulin receptor

(a) The amino acid sequences of the G-protein-binding motifs in insulin-like growth factor (IGF-II) [29] and β_2 -adrenergic (β_2 -Ad) [30] receptors and two putative G-protein binding peptides found in the insulin receptor (IR_{GPBP1} and IR_{GPBP2}) are shown. GPBP2_{R-Q1152} is a GPBP2 with a substitution of Q for R at 1152 (boxed). We have also synthesized a 22-amino-acid control peptide (CP), which is shown. The key basic residues (B) for protein binding are shown at the top, and X is any amino acid residue. Hyphens (-) are used to align amino acids which are not critical for G-protein binding. (b) Schematic representation of the insulin receptor β -subunit showing the location and amino acid positions [40] of the G-protein-binding sites, GPBP1 and GPBP2 and the control peptide (CP).

the insulin receptor β -subunit of these motifs and of a control peptide (CP) that does not fulfil the criteria for G-protein binding are shown in Figure 1(b).

Two mutations of the insulin receptor occurring in non-insulin-dependent diabetics have been identified in the GPBP2 region, one at residue Met $\rightarrow$ Ile¹¹⁴¹ [31] and the other at residue Arg $\rightarrow$ Gln¹¹⁵² (R-Q1152) [32]. Interestingly, the R-Q1152 mutation affects one of the key basic residues in the G-protein-binding sequence of GPBP2 (Figure 1). Insulin receptors with this mutation, prepared either from diabetic patients [32] or from NIH-3T3 cells transfected with the mutated insulin receptor [33], have an elevated basal kinase activity and lose insulin-sensitivity.

These observations led us to hypothesize that the 41 and/or 67 kDa G-proteins bind to one or both of the GPBP regions and modulate the insulin-receptor function. In the present study, we show that GPBP2 stimulates GTP[S] binding to the 67 kDa G-protein, providing a potential regulatory locus for the interaction of G-proteins with the insulin receptor.

EXPERIMENTAL

Materials

Antibody to common G α (GA/1) and GTP[³⁵S] were purchased from New England Nuclear (Boston, MA, U.S.A.). 8-Azido- α -³²P]GTP was obtained from ICN Biomedicals (Costa Mesa, CA, U.S.A.). Alkaline phosphatase linked to goat anti-rabbit IgG and the alkaline phosphatase developing kit were from Bio-Rad (Richmond, CA, U.S.A.). GTP[S], wheat-germ lectin–Sepharose 6MB and molecular-mass markers [thyroglobulin (669 kDa), alcohol dehydrogenase (150 kDa), BSA (66 kDa) and carbonic anhydrase (29 kDa)] used for size-exclusion chromatography were purchased from Sigma (St. Louis, MO, U.S.A.). Dialysis

membranes were from Spectrum (Houston, TX, U.S.A.). Immobilon-P was from Millipore (Bedford, MA, U.S.A.) and nitrocellulose membrane filters (BA85) used for the GTP[S] binding assay were from Schleicher and Schuell (Keene, NH, U.S.A.). Insulin was kindly given by Eli Lilly (Indianapolis, IN, U.S.A.). The Biosep SEC-S2000 (600 mm $\times$ 7.5 mm) size-exclusion column was from Phenomenex (Torrance, CA, U.S.A.). All other reagents were from standard sources.

Preparation of synthetic peptides

GPBP1, GPBP2 and a control peptide (CP) (see Figure 1) were synthesized by the solid-phase method and purified by h.p.l.c. as described in [34]. The freeze-dried peptides were dissolved in 6 M guanidinium chloride, dialysed (1 kDa cut-off) extensively against 50 mM NH₄HCO₃ and freeze-dried again until complete removal of NH₄HCO₃. A stock solution (10 mM) was prepared in 20 mM Tris/HCl, pH 7.4.

Preparation of insulin receptors and G-proteins associated with the insulin receptors

Partially purified insulin receptors were prepared from human placentas by wheat-germ lectin–agarose chromatography [22]. G-protein-depleted highly purified insulin receptors were prepared by insulin–Sepharose chromatography using the method of Fujita-Yamaguchi et al. [35] as modified by us [22]. The material that did not bind to the insulin–Sepharose [insulin–Sepharose Flow-through (ISFT)] contains the 41 and 67 kDa G-proteins that appear to be involved in insulin action [22]. ISFT was dialysed extensively against buffer A [20 mM Tris buffer (pH 8.0 at 4 °C)/150 mM NaCl/1 mM EDTA/1 mM dithiothreitol (DTT)/0.1% Triton X-100/1 mM phenylmethane-sulphonyl fluoride] and concentrated on a YM-10 membrane.

Purification of the 67 kDa G-protein

ISFT (25–35 mg), prepared from two human placentas, was incubated overnight at 4 °C with 40 ml of GDP–agarose slurry that had been equilibrated with 300 ml of buffer A. The slurry was transferred to a column and washed with 400 ml of buffer A. The bound material was eluted with 200 ml of buffer A supplemented with 2 mM GDP, concentrated to about 1 ml on a YM-10 membrane, dialysed extensively against buffer A and stored at –70 °C until used. This procedure was repeated seven times, with a typical yield of 0.5 mg from two placentas. The entire purification procedure was performed at 4 °C. The purity of the 67 kDa G-protein preparation was determined by silver staining (Bio-Rad kit) of an SDS/PAGE gel and by 8-azido- $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ cross-linking performed as described previously [22].

GTP[S] binding assay

In standard assays, ISFT (10–30 $\mu\text{g}/\text{assay}$) was preincubated for 30 min at 24 °C in 100 μl of assay buffer [20 mM Tris/HCl (pH 8.0)/100 mM NaCl/1 mM EDTA/1 mM DTT/0.1 % Triton X-100] with (non-specific binding) or without 1 mM unlabelled GTP[S]. Other additions are indicated in the Figure legends. GTP[S] binding was initiated by adding 1 μM GTP ^{35}S (2×10^4 c.p.m./pmol). After 1 h incubation at 30 °C, bound GTP[S] was measured by rapid filtration assay [36].

To isolate and identify GPBP2-stimulated GTP[S]-binding protein(s), ISFT was pre-incubated with 1 mM GPBP2 and bound to 1 μM GTP ^{35}S in 200 μl of assay buffer. A 50 μl aliquot was assayed by filtration to measure GTP[S] binding to ISFT. A 150 μl aliquot was chromatographed by isocratic h.p.l.c. on a Biosep size-exclusion column using the assay buffer as the mobile phase. Fractions (1 min) were collected at a flow rate of 0.6 ml/min. A 50 μl aliquot of each fraction was used to count GTP ^{35}S radioactivity and the remainder of the sample was precipitated with 10% trichloroacetic acid, separated by an SDS/10% PAGE and immunoblotted with a common G α antibody and alkaline phosphatase-linked to goat anti-rabbit IgG and colorimetrically developed as described in [22].

Other methods

The protein concentration was measured by the method of Lowry et al. [37], with BSA as standard. All values are expressed as means $\pm$ S.E.M.

RESULTS

GPBP2 stimulates GTP[S] binding to ISFT

We tested the effect of synthetic GPBP1 and GPBP2, which correspond to two putative G-protein-binding regions of the insulin receptor (Figure 1), on the ability of GTP[S] to bind to ISFT. Whereas GPBP1 had no effect on GTP[S] binding to ISFT, GPBP2 stimulated binding in a concentration-dependent manner (Figure 2a). The stimulatory effect of GPBP2 on the GTP[S] binding to ISFT was observed at as low as 75 μM GPBP2 (3.9 ± 0.4 -fold increase over ISFT alone; $n = 2$ different ISFT preparations). At 1 mM GPBP2, GTP[S] binding to ISFT was stimulated by 8.7 ± 1.8 -fold ($n = 8$ different ISFT preparations) (Figure 2a). To examine further the specificity of GPBP2 on ISFT, a 22-amino-acid control peptide (CP; see Figure 1) was used in the GTP[S] binding assay. As shown in Figure 2(b), 1 mM CP did not affect GTP[S] binding to ISFT,

whereas 0.5 mM GPBP2 stimulated GTP[S] binding to ISFT by 7.0 ± 0.6 -fold over the value obtained with ISFT alone. The stimulatory effect of GPBP2 on GTP[S] binding to ISFT was abolished by pretreating ISFT at 56 °C for 30 min (Figure 2b, bar 5).

The Arg (R) at position 1152 of the insulin receptor is not only a key residue forming the critical BBXXB motif of the GPBP2 region of the insulin receptor (Figure 1), but also is mutated to a Gln (Q) residue in some diabetics [32]. Therefore, we tested the effect of an R-Q1152 substitution (GPBP2_{R-Q1152}) on the GTP[S] binding to ISFT. When compared with 0.5 mM GPBP2, the stimulatory potency of 0.5 mM GPBP2_{R-Q1152} on GTP[S] binding to ISFT was attenuated by 58 % (Figure 2b, bar 6).

GPBP2-stimulated GTP[S] binding to ISFT was dependent upon increasing concentrations of GTP[S] and was saturable. When measured with 100 μM GPBP2, GPBP2-stimulated GTP[S] binding to ISFT reached steady state at 10 μM GTP[S]. The half-maximal concentration for binding was approx. 1 μM GTP[S] (Figure 3).

GPBP2 stimulates GTP[S] binding to the 67 kDa G-protein

To identify a protein or proteins responsible for GPBP2-stimulated binding of GTP[S] in ISFT, ISFT was first bound to

Figure 2 Effect of GPBPs on GTP[S] binding to ISFT

ISFT (10–30 μg), prepared as described in the Experimental section, was preincubated for 30 min at 24 °C with or without GPBPs as indicated and unlabelled 1 mM GTP[S]. GTP[S] binding was measured as described in the Experimental section. Specific binding activity was calculated by subtracting GTP[S] bound in the presence of 1 mM GTP[S] at each concentration of GPBPs. (a) is a summarized result of GTP[S]-binding activities measured in the presence of various GPBP concentrations using two to eight different ISFT preparations. To normalize the data, individual experiments were converted into fold stimulation compared with corresponding control values (ISFT alone). $\circ$, GPBP1; $\bullet$, GPBP2. (b) represents a summary obtained from two to four separate experiments. CP (1 mM), 0.5 mM GPBP2 or 0.5 mM GPBP2 (R-Q1152) were pre-incubated without (bars 1 and 3) or with (bars 2, 4 and 6) ISFT or heated ISFT (Heat, bar 5, 56 °C for 30 min) as indicated.

Figure 3 Saturability of GPBP2-stimulated GTP[S] binding to ISFT

ISFT (10 μ g) and 100 μ M GPBP2 were pre-incubated in the presence of increasing concentrations of unlabelled GTP[S] (0.1 nM–1 mM), and specific binding of GTP[35 S] was assayed as described in the Experimental section.

Figure 4 Identification of GTP[S]-binding protein(s) in ISFT by gel-filtration chromatography

GTP[S] binding to ISFT was performed in the presence of GPBP2, and the reaction products were separated by size-exclusion chromatography using h.p.l.c. as described in the Experimental section. A 50 μ l aliquot of each fraction was used to count GTP[35 S] radioactivity, and the remainder of the sample was precipitated with 10% (w/v) trichloroacetic acid, separated by SDS/PAGE and immunoblotted. (a) Elution profile of GTP[35 S] radioactivity, with the elution location of molecular-mass standards and free GTP[S] marked. (b) Immunoblot with a common $G\alpha$ antibody. The migrations of molecular-mass (M) standards and the 41 kDa and 67 kDa G-proteins are shown.

GTP[35 S] in the presence of 1 mM GPBP2 and the reaction products were separated by size-exclusion chromatography. The fractions were examined for GTP[35 S] radioactivity and immunoblotted with a common $G\alpha$ antibody. As shown in Figure 4(a), fractions 26, 27 and 28 contained the majority of bound GTP[35 S]. The estimated molecular mass of the peak fraction, 26, was 65 kDa. Immunoblot analysis with an

Figure 5 GPBP2 stimulates GTP[S] binding to the purified 67 kDa G-protein

The 67 kDa G-protein was purified from ISFT as described in the Experimental section. (a) Lane 1 ('GTP X-link') shows an autoradiogram obtained from 20 μ g of the purified 67 kDa G-protein that was cross-linked to 8-azido-[α - 32 P]GTP as described in the Experimental section; a silver stain of the same preparation is shown in the second lane ('Silver stain'). In contrast with the single protein band seen in the purified 67 kDa G-protein preparation, ISFT has approx. 11 bands (results not shown). (b) GTP[S]-binding activities of ISFT (30 μ g) or the purified 67 kDa G-protein (10 μ g) measured in the absence (–) or presence (+) of 0.5 mM GPBP2.

antibody to common $G\alpha$ revealed that the 67 kDa protein was most intensely stained in fractions 26, 27 and 28 (Figure 4b). The reason why the immunostaining recognized several bands at approx. 67 kDa area may be due to proteolysis of the 67 kDa G-protein during more than 2 h of assay at 24 $^{\circ}$ C or 30 $^{\circ}$ C. Although the 67 kDa G-protein was present in other fractions, its concentration was considerably lower than in fractions 26–28. A 41 kDa immunostained band occurred in fractions 23 and 24 only. We have previously identified the 41 and 67 kDa G-proteins associated with the insulin receptor in the ISFT prepared in the manner described here [22]. The 41 kDa G-protein reported here is similar to G_i -proteins, based on pertussis-toxin-catalysed ADP-ribosylation and immunoblot with G-protein antibodies [22]. G_i -proteins are heterotrimers composed of α -, β - and γ -subunits and are eluted as approx. 100 kDa protein complexes in a low-pressure size-exclusion chromatography [9]. The 41 kDa G-protein shown here was eluted close to a 150 kDa standard molecular-mass marker. Considering the different experimental conditions and columns used, the apparent difference in elution of these proteins may not be significant. The chromatographic data in Figure 4 suggested that the 67 kDa G-protein may be solely responsible for GPBP2-stimulated GTP[S] binding in the ISFT. To test this, we substituted highly purified 67 kDa G-protein for ISFT in the GTP[S]-binding assay.

The 67 kDa G-protein was purified to homogeneity from ISFT by GDP-agarose chromatography as described in the Experimental section. The resulting silver stain of the purified 67 kDa G-protein is shown in Figure 5(a) (lane 2). As previously reported, the 67 kDa G-protein bound 8-azido-[α - 32 P]GTP (Figure 5a, lane 1) and reacted to a common $G\alpha$ antibody (results not shown). If the 67 kDa G-protein were responsible for GPBP2-stimulated GTP[S] binding in the ISFT, then the purified protein would be able to substitute for ISFT in the GTP[S]-binding assay. In the absence of GPBP2, GTP[S] binding to ISFT was more than 2-fold higher than that to the purified 67 kDa G-protein (Figure 5b). In the presence of GPBP2, however, ISFT and the purified 67 kDa G-protein bound almost identical amounts of GTP[S] (Figure 5b). The purified 67 kDa G-protein was bound to GDP-agarose and subsequently eluted with 2 mM GDP (see the Experimental section). Therefore the purified

67 kDa G-protein was presumably saturated with GDP, which may have resulted in an underestimation of GTP[S]-binding activity. Nevertheless, the results shown in Figures 4 and 5 clearly demonstrate that GPBP2 stimulates GTP[S] binding to the 67 kDa G-protein.

DISCUSSION

We synthesized two peptides (GPBP1 and 2) that represent putative G-protein-binding sites in the cytosolic domain of the insulin receptor. These two GPBPs were selected on the basis of the criteria for G-protein-binding motif as shown by Okamoto et al. [29,30]. We have shown that GPBP2, but not GPBP1, stimulated binding of GTP[S] to an unpurified preparation of proteins derived from the insulin receptor (ISFT) in a concentration-dependent and saturable manner. The ISFT contains two G-proteins (41 and 67 kDa) that are derived from the insulin-receptor preparation [22]. GPBP2-stimulated GTP[S] binding to ISFT can be accounted for solely by the 67 kDa G-protein (Figures 4 and 5). These results indicate that although the G-protein-binding motif as defined by Okamoto et al. [29,30] may be essential for binding of the 67 kDa protein to the insulin receptor, other amino acids must also be important, since GPBP1 (which also contains the motif) did not stimulate GTP[S] binding.

Although the importance in insulin action of the interaction of the GPBP2 region of the insulin receptor and the 67 kDa G-protein is not clear without further studies, we speculate that the 67 kDa G-protein may act as a negative regulator maintaining the insulin receptor kinase in an unactivated or basal state. This speculation is supported by several lines of evidence. First, we have previously shown that removal of the 67 and 41 kDa G-proteins from the insulin receptor leads to an elevated basal receptor kinase activity and loss of insulin-sensitivity [22]. Reconstitution of the G-protein-depleted insulin receptor with a preparation containing the 67 kDa and 41 kDa G-proteins lowers the basal receptor kinase activity and partially restores insulin-sensitivity [22]. Addition of the highly purified 67 kDa G-protein alone inhibits insulin receptor tyrosine kinase activity of G-protein-depleted insulin receptors (H. Jo and J. M. McDonald, unpublished work). These findings suggest that binding of the 67 kDa G-protein with the insulin receptor may determine the basal kinase activity. Interestingly, the insulin receptor with the R-Q1152 mutation, which occurs at one of the key basic residues in the GPBP2 region, also has elevated basal kinase activity and is not insulin-sensitive [32,33]. GPBP2 with the R-Q1152 substitution peptide does not appear to interact with the 67 kDa G-protein as well as native GPBP2, as determined by its lower potency for stimulating GTP[S] binding. Finally, GTP[S] has been reported to inhibit insulin-receptor kinase activity [12,13]. It is conceivable, on the basis of our current and previous findings [22], that exogenously added GTP[S] binds to the 67 kDa G-protein which is coupled to the GPBP2 region of the insulin receptor. The GTP[S]-bound 67 kDa G-protein would maintain the receptor kinase in the basal state.

There are two interesting questions of immediate importance raised by these studies. One is the possible effect of tyrosine phosphorylation of GPBP2 on GTP[S] binding to the 41 and 67 kDa G-proteins. The GPBP2 region contains three tyrosine residues at 1146, 1150 and 1151, which become phosphorylated upon binding of insulin to the α -subunit of the insulin receptor [38]. Given the importance of autophosphorylation of these tyrosine residues in initiating the phosphorylation cascade stimulated by insulin, it will be especially interesting to study the interaction of phosphorylated GPBP2 with the 67 kDa and 41 kDa G-proteins. Another is the inability of GPBP2 to influence

GTP[S] binding to the 41 kDa G-protein in ISFT. One simple explanation is that the 41 kDa G-protein binds to a region of the insulin receptor other than GPBP1 or 2. Another possibility is that the 41 kDa G-protein may bind to the insulin receptor only when the receptor is activated. It is clear, however, that a 40/41 kDa G-protein is associated with the insulin receptor. Kellner et al. [13] have shown that insulin stimulates GTP cross-linking to a 40 kDa G-protein. We and others have shown that insulin inhibits pertussis-toxin-catalysed ADP ribosylation of a 41 kDa G-protein [22,39]. Furthermore, we (H. Jo and J. M. McDonald, unpublished work) and others [40] have been able to co-immunoprecipitate the insulin receptor with anti- $G_{i\alpha}$ antibody. In addition, insulin stimulates the amount of the insulin receptor co-immunoprecipitating with anti- $G_{i\alpha}$ antibody [40].

In summary, we report the identification of a G-protein-binding region in the tyrosine kinase domain of the insulin receptor. The synthesized GPBP2 stimulates GTP[S] binding to a 67 kDa G-protein derived from insulin-receptor preparations, indicating that the 67 kDa G-protein may be an important mediator of insulin-receptor function.

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