Conditional, Tissue-specific Expression of Q205L Ga\textsubscript{i2} \textit{in Vivo}
Mimics Insulin Activation of c-Jun N-terminal Kinase and p38 Kinase*

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Deficiency of the G-protein subunit Ga\textsubscript{i2} impairs insulin action (Moxham, C. M., and Malbon, C. C. (1996) \textit{Nature} 379, 840–844). By using the promoter for the phosphoenolpyruvate carboxykinase gene, conditional, tissue-specific expression of the constitutively active mutant form (Q205L) of Ga\textsubscript{i2} was achieved in mice harboring the transgene. Expression of Q205L Ga\textsubscript{i2} was detected in skeletal muscle, liver, and adipose tissue of transgenic mice. Whereas the Ga\textsubscript{i2}-deficient mice displayed blunted insulin action, the Q205L Ga\textsubscript{i2}-expressing mice displayed enhanced insulin-like effects. Glycogen synthase in skeletal muscle was found to be activated in Q205L Ga\textsubscript{i2}-expressing mice, in the absence of the administration of insulin. Analysis of members of mitogen-activated protein kinase family revealed that both c-Jun N-terminal kinase and p38 are constitutively activated \textit{in vivo} in the mice that express the Q205L Ga\textsubscript{i2}. ERK1,2, in contrast, are unaffected in the Q205L Ga\textsubscript{i2}-expressing mice. Insulin, like expression of Q205L Ga\textsubscript{i2}, activates both p38 and c-Jun N-terminal kinases as well as glycogen synthase. Activation of c-Jun N-terminal and p38 kinases \textit{in vivo} with anisomycin, however, was insufficient to activate glycogen synthase. Much like Ga\textsubscript{i2} deficiency provokes insulin resistance, expression of Q205L constitutively active Ga\textsubscript{i2} mimics insulin action \textit{in vivo}, sharing with insulin the activation of two mitogen-activated protein kinase members, p38 and c-Jun N-terminal kinases.

G-proteins mediate signals from a populous class of cell-surface membrane-bound receptors to a lesser group of effectors that includes adenylcyclases, phospholipase C\textsubscript{β}, and various ion channels (1, 2). Insulin receptors and other growth factor receptors with intrinsin tyrosine kinase activity, in contrast, phosphorylate substrate molecules that link directly or indirectly through adaptor molecules to pathways that include the mitogen-activated protein (MAP)\textsuperscript{3} kinase regulatory network (3, 4). Elucidation of the manner by which these two pathways integrate signaling poses formidable problems, and understanding of this integration is critical to cell signaling. Insights on the integration of signaling between G-protein-linked and tyrosine kinase-linked receptors have emerged. Signal integration can occur at the most proximal point, i.e. receptor-to-receptor. G-protein-linked receptors, e.g. the β-adrenergic and bradykinin receptors, have been shown to act as substrates for growth factor receptors with intrinsic tyrosine kinase activity (5, 6). The ability of insulin to phosphorylate these G-protein-linked receptors as well as the ability of insulin to counter-regulate catecholamine activation of adenyllylcyelase (6, 7) can be abolished through phenylalanine substitution of specific tyrosyl residues on the cytoplasmic, C-terminal domain of the β-adrenergic receptor.

G-proteins have been implicated as influencing insulin action. Inactivation of G-proteins with pertussis toxin alters insulin action in the liver (8), and treating cells with insulin induces changes in the accessibility of the G-protein that mediates the inhibitory control of adenyllylcyelase, denoted Ga\textsubscript{i2}, to antibody binding (9). Expression of RNA antisense to the pertussis toxin-sensitive Ga\textsubscript{i2}-subunit in selected target tissues of the mouse both eliminates Ga\textsubscript{i2} expression (10) and generates profound insulin resistance \textit{in vivo} (11). Ga\textsubscript{i2} deficiency provokes blunted glucose tolerance, loss of insulin-stimulated hexose transport, inability of insulin to recruit GLUT4 transporters to the plasma membrane, blunted glycogen synthase activation in liver and skeletal muscle of the transgenic mice, and enhanced expression of phosphotyrosine phosphatase 1b but not SYP (11). Conditional, tissue-specific targeting of expression of the constitutively active mutant form (Q205L) of Ga\textsubscript{i2} \textit{in vivo} has insulinomimetic effects (12). In the current work we investigate downstream elements of the mitogen-activated protein kinase pathways to explore the potential roles of ERK1,2, p38, and c-Jun N-terminal kinases in mediating the insulinomimetic effects of expression of Q205L Ga\textsubscript{i2} in transgenic mice.

MATERIALS AND METHODS

Creating Transgenic Mice with Conditional, Tissue-specific Expression of Q205L Ga\textsubscript{i2}—Details of the design of the vector and creation of mice harboring a transgene driving the expression of the constitutively active Q205L mutant of Ga\textsubscript{i2} are provided elsewhere (12). Briefly, the 1.7-kilobase pair cDNA harboring the constitutively active Q205L mutant of Ga\textsubscript{i2} (kindly provided by Dr. Gary L. Johnson, National Jewish Center, Denver, CO) was excised from the vector pCW1 and purified by agarose gel electrophoresis. The parental genomic PEPCK gene fragment used was a 7.0-kilobase pair EcoRI/BamHI fragment described previously (10). This genomic clone contains the elements necessary to maintain the developmental and tissue-specific expression of PEPCK mRNA as seen with the endogenous PEPCK gene. Prior to ligation with the Q205L Ga\textsubscript{i2} mutant cDNA, the PEPCK gene was digested with NheI (~11 with respect to transcription start site) and AatII (~4920) leaving the 5’ promoter and 3’-untranslated region of mRNA intact. HindIII linkers were then engineered at both the NheI and AatII sites to

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§ The abbreviations used are: MAP, mitogen-activated protein; GST, glutathione S-transferase; FPLC, fast protein liquid chromatography; PCK, phosphoenolpyruvate carboxykinase; PCR, polymerase chain reaction; JNK, c-Jun N-terminal kinase.
facilitate ligation with the Q205L Ga4 mutant. After successful ligation, the proper orientation of the Q205L Ga4 mutant cDNA was verified by restriction digest analysis. The transgene was excised as a 3.2-kilobase pair fragment using XhoI (5') and NotI (3') and prepared for a new round of microinjection into single cell, pre-implantation embryos for the production of transgenic mice for the disease (12). The mice were generated in the Transgenic Mouse Facility at Stony Brook. Mouse tail biopsies were obtained from the product litters, and DNA was isolated for PCR amplification with pPCK-Q205LGa4 transgene-specific primers, described elsewhere (12). Four founder lines were bred for 10 generations, and mice used in these experiments were 9–12 weeks of age, with no age-related differences observed in the parameters measured. All animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee at the State University of New York, Stony Brook. For each experiment reported animals from at least two founder lines were analyzed, samples being treated separately. In all cases, mice derived of each founder line displayed a uniform phenotypic character with respect to those parameters reported herein.

**Immunoblotting—** Samples of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 11% gels. The separated proteins were transferred electrophoretically to nitrocellulose blots and the blots subjected to immunoblotting (10).

**Glycogen Synthase Assay—** For determinations of glycogen synthase activity, skeletal muscle from the mice (1 g) was homogenized in 0.01 ml of perchloric acid (25% v/v) and centrifuged at 12,000 × g for 5 min at 4°C. The supernatant was then treated with 0.2 ml of 10% trichloroacetic acid (25% v/v) and the mixture was centrifuged at 12,000 × g for 5 min. The supernatant was then neutralized using a solution containing ketamine (120 mg/kg body weight) and xylazine (80 mg/kg body weight), injected intraperitoneally. Once deep anesthesia is achieved, the abdominal cavity is opened and the viscera exposed. Mice were administered either vehicle alone or insulin (10 units), via intravenous injection into the inferior vena cava. At the times indicated after injection, portions (200 mg wet weight) of the skeletal muscle (gastrocnemius) were removed and measurements of glycogen synthase activity performed as described previously (11). Protein determinations were performed according to Lowry et al. (13).

**Immunoblotting—** Aliquots of protein were subjected to 11% SDS-polyacrylamide gel electrophoresis, and the separated proteins were transferred electrophoretically from the gel to the nitrocellulose membrane. Antibodies to the following antigens employed in these studies were obtained from the indicated sources: JNK-1 (number SC-474-G, Santa Cruz Biotechnology, Santa Cruz, CA); ERK1 and -2 (numbers 61-740, Zymed Laboratories Inc., San Francisco, CA); dually phosphorylated, active ERK1 and -2 (number V6671, Promega, Madison, WI); p38 MAP kinase (number 06-394, Upstate Biotechnology, Lake Placid, NY); and dually phosphorylated (Thr-180 and Tyr-182), active p38 MAP kinase (number 9211S, New England Biolabs, Beverly, MA). In most cases, the immune complexes formed were made visible by using alkaline phosphatase-linked goat anti-rabbit IgG (Life Technologies, Inc.), rabbit anti-mouse IgG (Life Technologies, Inc.), or rabbit anti-goat IgG (Sigma). For antibodies against the dually phosphorylated, active forms of ERK1,2 and of p38 MAP kinase, the immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography, the bands for GST-Jun were excised and the incorporated 32P was quantified by liquid scintillation counting. Following FPLC fractionation, ERK1,2 are confined to fractions 4 and 5, whereas p38 is confined to fractions 7 and 8 (14).

**RESULTS AND DISCUSSION**

Mice harboring the pPCK-Q205L Ga4 transgene were identified by PCR analysis of tail DNA using unique primers (Fig. 1A). Shown are the PCR products of tail DNA from six members of a large litter in which animals 1–3 and 6 display amplification of the transgene. Mice identified as harboring the transgene (T) by PCR amplification expressed increased amounts of immunoreactive Ga4 (endogenous Ga4 and the Q205L Ga4) in fat (Fig. 1B), liver, and skeletal muscle (14). In each of these target tissues for the PCK promoter-driven expression, the levels of Ga4 expression were well in excess of that in the corresponding tissues of their control littermates (C). In non-target tissues, such as lung (Fig. 1B) as well as brain or spleen (not shown), the PCK promoter is not active, and expression of Ga4 is equivalent in transgenic (T) and control littermates (C) alike. Constitutive expression of Q205L Ga4 was persistent in all members of the four founder pPCK-Q205L Ga4 transgenic lines, each line propagated for more than 10 generations.

The activity ratio (−/+ Glut-6-P) of glycogen synthase for fasting mice in the absence of insulin (t = 0) was markedly greater in skeletal muscle from the mice harboring the pPCK-Q205L Ga4 transgene than in their FVB control littermates (Fig. 2). In fact, the levels of activation of glycogen synthase observed in skeletal muscle of the fasted, pPCK-Q205L Ga4 mice is comparable to that observed in skeletal muscle of control mice treated with insulin in vivo for 15 min. The activity ratios for skeletal muscle glycogen synthase in control mice are 0.25 ± 0.001 without insulin and 0.39 ± 0.03 (n = 6) following intravenous administration of insulin. These data agree well with earlier results of hepatic glycogen synthase activation, glucose tolerance tests, analysis of hexose transport, and the data on GLUT4 recruitment (12). The elevated levels of glycogen synthase activation in skeletal muscle of the pPCK-Q205L Ga4 mice observed in vivo in the absence of insulin administration provide compelling evidence that expression of Q205L Ga4 in vivo is insulinomimetic with respect to activation of glycogen synthase in skeletal muscle.

Earlier we elucidated a signal linkage map of the control of glycogen synthase activation in the BDF1 strain of mice (14) at the levels of mitogen-activated protein kinases (MAP kinases),
ribosomal S6 kinase, and glycogen synthase kinase-3. ERK1,2 has been implicated in downstream signaling by several growth factors, including insulin. To address the possible role of ERK1,2, activation was assessed using phospho-specific antibodies that recognize only the dually phosphorylated, fully active form of the kinases. When administered to mice in vivo, insulin stimulated activation of ERK1,2 (Fig. 3). The activation of ERK1,2 peaks at about 8 min following insulin injection into the vena cava (Fig. 3). Activation of glycogen synthase, in contrast, is absent at 2, 4, and 8 min postinsulin administration in vivo, occurring between 8 and 16 min postinsulin in the FVB strain of mice. Glycogen synthase activity was measured in skeletal muscle but has little effect on the activity of ERK1,2. These data obtained in FVB mice support earlier temporal studies of insulin action per-

To address if activation of ERK1,2 was mediating the constitutive activation of glycogen synthase in the mice harboring the pPK-Q205L Gαi2 transgene, the activity of ERK1,2 was measured both in skeletal muscle control and of transgenic mice, after the mice have been fasted overnight. Although glycogen synthase is fully activated in the fasted mice expressing the Q205L Gαi2, ERK1,2 activity actually displayed a slight, although non-significant, reduction rather than marked elevation in the transgenic mice compared with their control littermates, i.e., epidermal growth factor receptor peptide phos-

![Image](73x568 to 273x729)

**FIG. 1.** Expression of the constitutively active Q205L Gαi2 by the promoter for the PEPCK gene in target tissues of transgenic mice. A, identification of mice harboring the pPK-Q205L Gαi2 transgene by PCR. Mouse tail DNA was isolated and amplified using primers unique to the transgene. The lanes of the ethidium bromide-stained agarose gels are as follows: molecular markers (M); negative control without added DNA (NC); positive control, amplification from the pPK-Q205L Gαi2, and amplification of tail DNA from six members of one litter in which animals 1–3 and 6 score positive for the presence of the transgene. Details of the analysis is provide elsewhere (10–12, 14). B, expression of Q205L Gαi2 is induced in a tissue-specific manner in mice harboring the pPK-Q205L Gαi2 transgene. Crude membranes were prepared from a target tissue (Fat) as well as a non-target tissue (Lung) of mice harboring the pPK-Q205L Gαi2, or their control littermates. Crude membrane protein (10 and 20 μg/lane/SDS gel) was subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting. The lanes of the ethidium bromide-stained gels are as follows: molecular markers (M); negative control (C) and crude membranes (T) mice, respectively: fat, 0.79 and 3.42 (shown); lung, 1.58 and 1.79 (shown); and other tissues not shown, including liver, 0.3 and 0.7; brain, 1.83 and 1.85; and spleen, 1.50 and 1.11. Target tissues for expression of the pPK-Q205L Gαi2 transgene include fat, liver, and skeletal muscle (10–12, 14). Tissues isolated from transgenic mice derived from each founder line all displayed increased expression of immunoreactive Gαi2 ranging from 1.6- to 2.2-fold in fat tissue and from 1.4- to 2.6-fold in liver tissue (14) when compared with the expression of immunoreactive Gαi2 in control littermate tissues.

![Image](337x503 to 525x729)

**FIG. 2.** Glycogen synthase is activated in transgenic mice with targeted expression of the constitutively active Q205L Gαi2 in vivo. After an overnight fast, pPK-Q205L Gαi2 transgenic mice displayed hypoglycemia relative to their control littermates. pPK-Q205L Gαi2 transgenic mice display enhanced tolerance to challenge with glucose. Glycogen synthase activity was measured in skeletal muscle of control and transgenic mice at t = 0 and at 16 min following administration of insulin in vivo, as described under “Materials and Methods.” The results shown are the mean values ± S.E. from 6 mice for each condition. * denotes values statistically different from control (p < 0.05).

![Image](349x503 to 513x729)

**FIG. 3.** Activation of ERK1,2 by insulin in skeletal muscle in vivo. Time course of the activation of ERK1,2 in skeletal muscle of FVB mice. Mice were administered insulin in vivo, and the extent of activation of ERK1,2 was measured using phospho-specific antibodies that recognize only the dually phosphorylated, activated form of ERK1,2. The results presented are the mean values ± S.E. (n = 6). * denotes values significantly different from control (p < 0.05).
formed in BDF1 mice suggesting that activation of ERK1,2 is not an important element of glycogen synthase activation by insulin in skeletal muscle (14).

The status of c-Jun N-terminal kinase (JNK) was assessed, since activation of glycogen synthase by insulin is accompanied by and perhaps mediated via activation of JNK in BDF1 mice (14). JNK activity was measured by the solid phase assay using a GST-c-Jun fusion peptide as the substrate. Administration of insulin in vivo results in a rapid and robust activation of JNK (Fig. 4). Activation of JNK in response to insulin was observed as early as 30 s following insulin administration in vivo, far more rapid in response than the activation of ERK1,2. Maximal activation of JNK is achieved within 2 min following administration of insulin. JNK activation is increased approximately 5-fold over basal at 2 min postinsulin.

**FIG. 4. Activation of JNK by insulin in skeletal muscle in vivo.** Time course for the activation of JNK in skeletal muscle of FVB mice following administration of insulin in vivo, using GST-Jun fusion protein as the substrate for JNK phosphorylation. The results shown are the mean values ± S.E. (n = 6). * denotes values significantly different from control (p < 0.05).

**TABLE I**

| Animal       | Tissue            | Insulin | Time (min) | Assay | Fold activation |
|--------------|-------------------|---------|------------|-------|----------------|
| FVB control  | Skeletal muscle   | No      | 0          | JNK   | 1.0 ± 0.09     |
| FVB control  | Skeletal muscle   | Yes     | 2          | JNK   | 3.5 ± 0.53*    |
| Q205L mice   | Skeletal muscle   | No      | 0          | JNK   | 3.2 ± 0.67*    |
| Q205L mice   | Skeletal muscle   | Yes     | 2          | JNK   | 3.5 ± 0.38*    |

Basal activity of skeletal muscle JNK is increased 3-fold in the mice expressing the Q205L Ga12 (Table I), following an overnight fast. The activity of JNK in the unstimulated, fasted transgenic mice is indistinguishable from the level of JNK activation observed in the fasted FVB control littermates following challenge with insulin. Of interest, administration of insulin to the Q205L transgenic mice fails to increase appreciably the level of JNK activity over that observed in the basal, unstimulated state, i.e. t = 0 for insulin administration. Constitutive activation of JNK activity is not confined to skeletal muscle; measurement of JNK in fat tissue likewise displays constitutive activation of JNK in the fasted transgenic mice (Table I). Fat and skeletal muscle are target tissues for expression of the pPCK-Q205L Ga12 transgene (10–12). In spleen and heart, which are non-target tissues for expression of the pPCK-Q205L Ga12 transgene, basal JNK activity of the transgenic mice is equivalent to the values obtained with these tissues obtained from the FVB control mice (Table I). These data demonstrate not only tissue-specific expression of the pPCK-Q205L Ga12 transgene but also expression in the target tissues does not lead to a pleiotropic activation of JNK at non-target sites. More importantly, the constitutive activation of JNK in the skeletal muscle and fat of mice expressing the constitutively active Q205L Ga12 suggests strongly that JNK is a likely contributor to the insulinomimetic effects observed in the transgenic mice.

The protein synthesis inhibitor anisomycin has been reported to activate selective members of MAP kinase family, at concentrations less than that necessary to block protein synthesis (16–18). Although a non-physiological agent, anisomycin was tested for its ability to activate JNK activity of skeletal muscle in vivo. Administration of anisomycin (20 mg/kg body weight) in vivo provoked activation of JNK activity in skeletal muscle (Fig. 5) but failed to activate appreciably glycogen synthase within 16 min of administration. The activity ratio (−glucose-6-phosphate/+glucose-6-phosphate) was unchanged from the 0 time point (0.26 ± 0.1) at either 8 min (0.26 ± 0.2) or 16 min (0.28 ± 0.6) following administration of 20 mg/kg body weight of anisomycin (mean ± S.E., n = 3). These observations in FVB mice are very different from those obtained with the mice of the BDF1 strain, which display a rapid activation both of JNK and of glycogen synthase in response to administration of anisomycin in vivo. Taken together, the time course of JNK...
activation and its persistent activation in the mice expressing the constitutively active form of Q205L Ga\(_{i2}\) suggest that activation of JNK alone may be insufficient to activate glycogen synthase.

p38 is a newly discovered member of the MAP kinase family shown earlier to be a novel feature of insulin signaling in some cells in culture (15). We explored the ability of insulin to activate p38 kinase in control FVB mice administered insulin in vivo, using antibodies that recognize only the dually phosphorylated, fully active form of p38 (Fig. 6). Insulin stimulates a rapid and robust activation of p38 kinase in skeletal muscle. Activation of p38 is measurable within 1 min and peaks at 4 min following challenge with insulin in vivo. A ~3.5-fold increase in the amounts of active p38 is observed at 4 min postinsulin challenge. These data provide the first demonstration of a rapid, robust activation of p38 kinase in vivo in a major target tissue for insulin action, i.e. skeletal muscle.

Targeted expression of the Q205L Ga\(_{i2}\) provokes constitutive activation of p38 kinase in the fasted, transgenic mice in the absence of insulin (Table II). The level of p38 kinase activation in the skeletal muscle of fasted transgenic mice is approximately 60% of that observed in the skeletal muscle of control littermates administered insulin. Challenging the Q205L Ga\(_{i2}\)-expressing mice with insulin, however, produces no significant increase in the amount of active p38 in skeletal muscle compared with that constitutively activated in the basal state of these mice (Table II). By using a GST-ATF2 fusion protein as the substrate for measurements of activities in tissue extracts subjected to FPLC separation, we tested the results obtained with the phospho-specific antibodies to p38. Direct assays of p38 activity in extracts of skeletal muscle show constitutive activation.

**FIG. 5.** Anisomycin activates skeletal muscle JNK in vivo. Mice were administered anisomycin (20 mg/kg) via the vena cava, and the activation of JNK was measured at the times indicated over the next 16 min. JNK activity was measured using the phosphorylation of GST-Jun fusion protein (inset), quantified from three or more independent determinations. The results shown are the mean values ± S.E. (n = 3). * denotes values significantly different from control (p < 0.05).

**FIG. 6.** Activation of p38 kinase by insulin in skeletal muscle in vivo. Time course of the activation of p38 kinase in skeletal muscle of FVB mice following administration of insulin in vivo, using phospho-specific antibodies that recognize only the dually phosphorylated, active form of p38. The results shown are the mean values ± S.E. (n = 6). * denotes values significantly different from control (p < 0.05).

**TABLE II**

| Animal   | Tissue       | Insulin | Time (min) | Assay       | Fold activation |
|----------|--------------|---------|------------|-------------|----------------|
| FVB control | Skeletal muscle | No    | 0          | Phospho-p38 | 1.0 ± 0.07     |
| Q205L mice | Skeletal muscle | Yes  | 4          | Phospho-p38 | 2.8 ± 0.48*    |
| Q205L mice | Skeletal muscle | Yes  | 4          | Phospho-p38 | 2.1 ± 0.27*    |

| Animal   | Tissue       | Insulin | Time (min) | Assay       | Fold activation |
|----------|--------------|---------|------------|-------------|----------------|
| FVB control | Skeletal muscle | No    | 0          | p38 activity | 1.0 ± 0.11     |
| Q205L mice | Skeletal muscle | Yes  | 4          | p38 activity | 2.3 ± 0.51*    |
| Q205L mice | Skeletal muscle | Yes  | 4          | p38 activity | 2.0 ± 0.47*    |

**p38 kinase activation is enhanced in skeletal muscle of transgenic mice with targeted expression of the constitutively active Q205L Ga\(_{i2}\)**

The time course of the activation of p38 kinase in skeletal muscle of FVB mice following administration of insulin in vivo by using phospho-specific antibodies that recognize only the dually phosphorylated, active form of p38 is displayed in Fig. 6. Top, activation of p38 kinase by insulin in skeletal muscle of control FVB mice and mice with targeted expression of the constitutively active Q205L Ga\(_{i2}\) by using phospho-specific antibodies that recognize only the dually phosphorylated, active form of p38. Bottom, activation of p38 kinase in skeletal muscle of control FVB mice and mice with targeted expression of the constitutively active Q205L Ga\(_{i2}\), measured using GST-ATF2 fusion protein as a substrate for p38 kinase phosphorylation. The results shown are the mean values ± S.E. (n = 6). * denotes values significantly different from control (p < 0.05).
activation of p38 in the mice expressing the Q205L mutant form of Ga12 (Table II). Tissue extracts from fat and skeletal muscle of both control and transgenic mice that were subjected to FPLC fractionation and to direct assay of p38 activity measured in the resolved fractions also reveal a constitutive, robust activation of p38 kinase in the mice expressing the constitutively active Q205L Ga12 (Fig. 7, A and B, for fat and skeletal muscle, respectively). Measured either by phospho-specific antibodies or by direct measure of activity using an GST-ATF2 fusion protein as substrate, p38 kinase is shown to be activated temporally by the administration of insulin in vivo and constitutively in those mice expressing the mutant Q205L Ga12 in a tissue-specific manner (Fig. 7 and Table II).

Anisomycin administration in vivo activates p38, as determined either by immunoblotting with phospho-specific antibodies to the dually phosphorylated, fully active form of the kinase (Fig. 8). Results of direct measurement of p38 kinase activity using the ATF-2 GST fusion protein as a substrate confirm these data, showing a 3.0 ± 0.1 (mean ± S.E., n = 3)-fold increase in activity at 6 min following administration of anisomycin in vivo. Thus, anisomycin administration in vivo provokes an activation of both JNK and p38 but fails to activate glycogen synthase. Mice expressing the constitutively active Q205L Ga12, in contrast, display constitutive activation of p38, JNK, and glycogen synthase.

The first signal linkage map prepared from analysis of insulin action in vivo suggests a possible central role of JNK in the activation of glycogen synthase (14). The temporal relationship between the activation of ERK1,2 and of JNK with downstream elements p90Rsk and PP-1 in vivo suggests that JNK can mediate activation of glycogen synthase by insulin (14). Activation of ERK1,2 in skeletal muscle of BDF1 mice follows rather than precedes the activation of glycogen synthase, whereas JNK activation precedes the activation of glycogen synthase. In addition, administration of anisomycin in vivo provokes robust activation both of JNK (but not ERK1,2) and of glycogen synthase (14).

In the present work, a rather different pattern of MAP kinase activation was observed in response to insulin administration in vivo. Of the three MAP kinase family members tested, ERK1,2 displays the slowest time course for activation in response to insulin in FVB mice, as was the case in BDF1 mice as well (14). JNK (and p38) are activated rapidly by insulin, again much like the response observed earlier in the BDF1 mice (14). Since the activation of glycogen synthase follows the activation of both JNK and p38 kinases by insulin, one or both of these kinases are candidate mediators of insulin action on glycogen synthase. Although anisomycin clearly activates skeletal muscle JNK and p38 in the FVB as well as the
BDF1 mice, the FVB mice fail to display activation of glycogen synthase in response to anisomycin administration in vivo. p38, the newest member of the MAP kinase family to be identified, is activated rapidly in vivo by insulin in skeletal muscle. In the absence of specific activators and inhibitors of p38 for use in vivo, the role of p38 kinase as a possible mediator for glycogen synthase activation in response to insulin in vivo at present cannot be defined more precisely. Support for a role of p38 in insulin action in the skeletal muscle of FVB mice was garnered through the analysis of the transgenic mice, harboring the Q205L Gaα2. Previously, it was shown that tissue-specific deficiency in Gaα2 results in frank insulin resistance in mice harboring the pCKASGaα2 antisense-producing transgene (10–12). Expression of the constitutively activated form of Q205L Gaα2, in contrast, enables transgenic mice to tolerate high glucose loading much better than control littersmates (12). Expression of the Q205L Gaα2 results in constitutive activation of glycogen synthase and appears insulinomimetic with regard to glucose transport, glucose transporter (GLUT4) recruitment, and glucose metabolism (12). In the current work we explored the status of three members of the MAP kinase regulatory network in order to determine the level to which the effects of insulin as well as the seemingly insulinomimetic effects of Q205L Gaα2 were being expressed in the transgenic mice.

Both p38 and JNK are constitutively activated in mice expressing Q205L Gaα2, demonstrating their downstream role in signaling via this heterotrimeric G-protein in vivo. JNK alone was suggested to be a mediator of insulin action on glycogen synthase in BDF1 mice (14), but these earlier studies were performed before p38 kinase was revealed as a member of the MAP kinase family. Although constitutively activated in the Q205L Gaα2-expressing mice, JNK and p38 are activated rapidly and robustly by anisomycin, but this activation is insufficient to stimulate activation of glycogen synthase. Either the temporal features or the magnitude of activation of p38 and JNK by insulin in glycogen synthase activation may be poorly duplicated by anisomycin. Alternatively, some additional signaling element, such as protein kinase B (19, 20), in addition to activation of p38 and of JNK may be lacking from the anisomycin response that is present during activation either by insulin or by expression of Q205L Gaα2 in vivo. Activation of protein kinase B has been shown to promote glycogen synthesis in L6 myotubes but not 3T3-L1 mouse adipocytes in culture (20). Clearly insulin action remains multifaceted. In skeletal muscle the relationship between changes in activation of JNK and of p38 correlates with activation of glycogen synthase in response to either insulin administration in vivo or expression of Q205L Gaα2 in vivo. Further analysis will be required to establish the precise linkage(s) between members of the MAP kinase family and insulin action in skeletal muscle and other insulin-sensitive tissues.

Expression of constitutively active Q205L Gaα2 in vivo mimics many facets of insulin action, revealing a provocative similarity between signaling via heterotrimeric G-proteins and signaling via growth factor receptors with intrinsic tyrosine kinase. Heterotrimeric G-protein signaling culminating in the activation of MAP kinases has been reported. m2 muscarinic receptors have been shown to activate ERK1,2, presumably to reflect a result of the release of βγ subunits from activated Gaα1 (21). In skeletal muscle, however, expression of Q205L Gaα2 in vivo resulted in no activation of ERK1,2. In human embryonal kidney cells, p38 kinase can be activated by expression of constitutively activated Gaα1 but not by expression of Q205L Gaα2 (22). Acting through Gaα2, JNK has been shown to be activated by stimulation of muscarinic acetylcholine receptors in Rat-1 cells (23, 24). In vivo, expression of Q205L Gaα2 in skeletal muscle and fat tissue activates both p38 kinase and JNK constitutively. The fact that ERK1,2 is not activated in the transgenic mice expressing the Q205L Gaα2 argues against its role as well as a role for βγ subunits in the physiology under study here. Clearly the ability to probe the functional role of Gaα2 in vivo in transgenic mice via expression of the Q205L Gaα2 provides a novel strategy to compare that which we deduce from in vitro studies of G-proteins and MAP kinases signaling to the in vivo arena. In addition, this works enables us to explore the role of Gaα2 in insulin action from a rather unique perspective, i.e. constitutive activation of Gaα2 is insulinomimetic, whereas Gaα2 deficiency has been shown to provoke insulin resistance (10–12).

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