Growth Hormone-dependent Differentiation of 3T3-F442A Preadipocytes Requires Janus Kinase/Signal Transducer and Activator of Transcription but Not Mitogen-activated Protein Kinase or p70 S6 Kinase Signaling*

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The differentiation of adipocyte precursor cells (preadipocytes) into mature adipocytes involves a coordinated program of gene induction and repression, which is under the influence of hormonal, neural, and dietary signals (1, 2). A major regulator of this process is growth hormone (GH),* which has been shown in several studies to promote the differentiation of preadipocytes both in vitro and in vivo (3–9). The dual effector theory has been proposed to explain the actions of GH upon differentiation of preadipocyte cells (3). GH is thought to induce a primed state in the preadipocytes (Gp), in which cells acquire increased responsiveness to insulin and insulin-like growth factor 1 (IGF-1), which then promote terminal differentiation. However, the intracellular signaling mechanisms underpinning these actions have not been established. We have been studying the differentiation of 3T3-F442A preadipocytes, which is dependent on both GH and insulin, with other factors exerting a modulatory influence (6). Recent work from several laboratories has begun to define the signaling pathways induced by GH in various cell types. GH induces activation of the non-receptor tyrosine kinase JAK-2, an event that is believed to initiate multiple downstream signaling pathways including activation of STAT transcription factors and the MAP kinase and p70(S6K) cascades (reviewed in Ref. 10). However, the roles played by these signals in biological actions of GH, such as the promotion of preadipocyte differentiation, have not been fully elucidated. Studies in this area have been hampered by the lack of a system allowing the action of GH to be studied in the absence of other extracellular factors. The development of serum-free media capable of supporting adipocyte differentiation has permitted the analysis of specific combinations of agents on the process (6, 7). In this study, we describe a serum-free protocol for the differentiation of 3T3-F442A cells in which the priming effects of GH can be separated from the actions of other factors that subsequently induce terminal differentiation. This system has been used to investigate the importance of JAK-2 and the STAT, MAP kinase, and p70(S6K) cascades in regulating GH-induced priming of 3T3-F442A preadipocytes.

EXPERIMENTAL PROCEDURES

Materials—Culture media, newborn calf serum, fetal calf serum, Lipofectin, and epidermal growth factor (EGF) were obtained from Life Technologies Inc. (Grand Island, NY). Transferrin, insulin, dexamethasone, 3-isobutyl-1-methylxanthine, and forskolin were obtained from Sigma Chemical Co. (St. Louis, MO). Epidermal growth factor (EGF), triiodothyronine (T3), and insulin were obtained from Collaborative Research Inc. (Lexington, MA). Oligodeoxynucleotides (ODNs) were synthesized by the DNA synthesis facility of the University of Manchester. Oligonucleotides were annealed in 100 mM NaOH and then neutralized with 0.5 M Tris base. Transfection reagents included LipofectAMINE (Gibco BRL Life Technologies, Inc.) and Lipofectin (Life Technologies Inc.). For transfection experiments, serum-free Medium 199 (M199) supplemented with 5% dialyzed calf serum was used. Protein kinase inhibitors used were 10 mM Sodium orthovanadate, 10 mM H89, 100 nM SB203580, and 10 μM 5,6-Dichloro-1-b-D-ribofuranosylbenzimidazole (DBC). The compounds were dissolved in dimethyl sulfoxide and added to cell cultures and media at a concentration of 10 μM.

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* The abbreviations used are: GH, growth hormone; MAP, mitogen-activated protein; STAT, signal transducer and activator of transcription; IGF-1, insulin-like growth factor-1; EGF, epidermal growth factor; T3, triiodothyronine; ETI, EGF, T3, and insulin; GPDH, α-glycerol-3-phosphate dehydrogenase; ODN, oligodeoxynucleotide; DMEM, Dulbecco’s Modified Eagle’s Medium; DDM, defined differentiation medium (standard serum-free medium (F12:DMEM, 2:1) plus 10 μg/ml transferrin, 50 μg/ml fetuin, 2.5 mM glutamine, and 1 mg/ml bovine serum albumin containing 2 mM GH, 1.8 μM insulin, 0.1 ng/ml T3, and 50 ng/ml EGF; JAK, Janus kinase.
Preadipocyte Differentiation Signaling

Technologies, Inc. Tri-iodothyronine (T₃) and insulin were obtained from Sigma. PD 098059 and rapamycin were obtained from Calbiochem. Recombinant bovine GH was a gift from Monsanto (St. Louis, MO). The scrambled sequence 5’-GCTTGTGAGAAAGC-3’ and the monomeric antibody to p42MAPK, p70e65/p85e5, and JAK-2 have been described previously (11). Rabbit antiserum to recombinant bovine GH (anti-rabbit GH) (12) and the monoclonal antibody to p42MAPK were generous gifts from Dr. D. J. Flint (Hannan Research Institute, Ayer, Scotland) and Prof. Ailsa Cambell (Institute of Biological and Life Sciences, University of Glasgow, Glasgow, Scotland), respectively. Donkey anti-serum to rabbit IgG was from the Scottish Antibody Production Unit (Carlisle, Scotland). IgG-hors eradish peroxidase conjugate was obtained from Amersham plc. Anti-rabbit IgG-horseradish peroxidase conjugate was obtained from Sigma. Antiseria to STAT-1, STAT-3, and STAT-5 were from Transduction Laboratories (Lexington, KY).

Standard Procedure for Cell Culture and Differentiation—3T3-F442A cells (Dr. Howard Green, Harvard Medical School) were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% newborn calf serum. Confluent cultures were washed three times in phosphate-buffered saline and incubated in a defined differentiation medium (DDM) (13). Under these conditions, at least 70% of the cells expressed adipocyte morphology and were positive for Oil Red O staining after 6–10 days.

Phase Protocol for Cellular Differentiation—For these studies, cells were grown to confluence in the presence of 10% newborn calf serum that had been depleted of GH. This was prepared by incubating the serum with anti-rGH (1:1000 dilution) for 24 h at room temperature. Antiserum to rabbit IgG was then added to a final dilution of 1:10. After 4 h, the precipitate was pelleted by centrifugation at 3000 × g for 30 min, and the supernatant (GH-depleted calf serum) was carefully removed. Cells were washed at least twice in medium containing GH-depleted calf serum before use for differentiation studies. Confluent cultures were washed three times in phosphate-buffered saline and then incubated for 2 days in standard serum-free medium (see above) with or without 2 mM GH. Cultures were then washed, as before, and the medium was replaced with maturation medium (standard serum-free medium containing 1.6 μM insulin, 50 ng/ml EGF, and 0.1 ng/ml T₃). Differentiation was measured after an additional 6–8 days.

Immunoblotting—Cells were washed once in ice-cold phosphate-buffered saline and then lysed at 4 °C in Buffer A (25 mM HEPES, pH 7.5, 50 mM NaCl, 2.5 mM EDTA, 50 mM NaF, 30 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 2 mM/ml aprotinin, 2 mM/ml pepstatin A, 2 mM/ml leupeptin, 10% (v/v) glycerol, and 1% (v/v) Nonidet P-40), clarified by centrifugation (14,000 rpm, 10 min), and denatured by adding 0.25 volume of 5X concentrated sample buffer. Equal quantities of lysate protein (10–25 μg) were electrophoresed on polyacrylamide gels as follows: MAP kinases, 10% (final acrylamide concentration), 30:0.32 (acylamide:bis-acrylamide ratio); p70e65, 9%, 30:0.32; and phosphotyrosine: 8%, 30:0.8. After transferring to nitrocellulose, immunoblots were developed in 3% (w/v) bovine serum albumin with rabbit anti-anti-phosphotyrosine (1:1000), anti-p70e65 (1:1000), or anti-phosphophorysine (1:1000) antibodies for 3 h followed by horseradish peroxidase-conjugated anti-IgG (1:10000) for 45 min. Immunoreactive bands were visualized by chemiluminescence using the ECL (Amersham) system.

Immunoprecipitation—JAK-2 and STAT-5 were detected after their immunoprecipitation and the subsequent immunoblotting of cells using anti-phosphotyrosine antibodies (PY99; Santa Cruz) as described previously (11).

Oligodeoxynucleotide (ODN) Treatment of Cells—The design, synthesis, and purification of MAP kinase phosphoantibodies ODNs have been described previously (13). The oligodeoxynucleotide 5’-GCCCTTGCAGAAAGC-3’ that is complementary to nucleotides 1902–1915 in murine JAK-2. The complementary sense ODN had the sequence 5’-GCCCTTGCACAAGG-3’. The JAK-2 control ODN had the scrambled sequence 5’-TCAGGCAATGTGGAG-3’. The antisense ODN to STAT-5 had the sequence 5’-AGGCCGCCCAT-3’, and the complementary sense ODN had the sequence 5’-ATGCGGGGCT-3’. The scrambled STAT-5 control ODN had the sequence 5’-CACCTGCACAC-3’. Cells in 22-mm-diameter wells were grown to 80–90% confluence before treatment. Appropriate dilutions of ODNs in 100 μl of DMEM were preincubated at room temperature for 30 min with 100 μl of Lipofectin (200 μg/ml). Monolayers were washed three times with 2 ml of DMEM, and the ODNs were added to the cells together with an additional 200 μl of DMEM. The final concentration of ODN was 5 μM. Cells were incubated for 8 h at 37 °C. After this time, the medium was removed, and the incubation was continued for an additional 40 h using fresh medium containing 10% heat-inactivated calf serum and 5 μM ODN but no Lipofectin. The medium was then removed, and cells were either harvested immediately or washed and subjected to the standard differentiation procedure in DDM containing 5 μM ODN, as described above. The differentiation medium was replenished at 2-day intervals, and ODN was present throughout the differentiation procedure.

Microscopy—Cells were photographed under Phase Contrast Optics (×24 magnification) using an Olympus IMT2 inverted microscope. a-Glycerol-3-Phosphate Dehydrogenase (GPDH) and DNA Assays and Expression of Results—GPDH activity was measured by the method of Wise and Green (15). In accordance with others (16), we found that the amount of protein per cell was increased by a factor of ~2.3 in adipocytes compared with fibroblasts. GPDH activities are therefore expressed relative to DNA content rather than the amount of cellular protein. The DNA content of cellular homogenates was determined fluorometrically (17). The enzyme activity per plate of cells was expressed as the number of micromoles of NADH oxidized/min (unit/mg DNA). Statistical significance was assessed by Student’s t test.

RESULTS

Previous work has shown that differentiation of 3T3-F442A preadipocytes can be induced in a serum-free medium (6, 13). GH, insulin, and EGF are essential components of this medium, with other factors exerting modulatory influences. In order to study GH action independently, we developed a two-step protocol (described under “Experimental Procedures”) for differentiation of 3T3-F442A preadipocytes. Cells were grown to confluence in the presence of serum depleted of GH and then primed for 48 h with or without GH. Cells were then exposed to EGF, T₃, and insulin (ETI) to induce terminal differentiation that was quantitatively assessed by measuring the cellular activity of the adipocyte-specific marker GPDH and qualitatively assessed morphologically (Fig. 1). These data show that cells must be primed with GH before the induction of terminal differentiation by ETI and that terminal differentiation is GH-independent. Because IGF-1 is known to mediate many of the biological effects of GH (18), we tested whether IGF-1 alone could prime cells for differentiation. Fig. 1 shows that, although pretreatment of cells with IGF-1 promoted the subsequent terminal differentiation to a degree, the magnitude of this effect was much less than that induced by GH. This indicates that the priming effect of GH is primarily due to direct actions of the hormone.

Cellular Depletion of JAK-2 Blocks GH-dependent Differentiation—Activation of the non-receptor tyrosine kinase JAK-2 is considered to be essential for GH-dependent signal transduction (19–21). To test whether JAK-2 is necessary for GH-induced priming of preadipocytes, we used an antisense ODN to deplete JAK-2 from the cells. Preadipocytes, which had been grown to confluence in GH-depleted medium to ensure that subsequent differentiation took place under GH-dependent conditions, were treated with the JAK-2 antisense ODN before the induction of differentiation. The extent of differentiation was assessed by measurement of GPDH activity. These experiments revealed that depletion of cellular JAK-2 almost completely blocked the subsequent adipogenic differentiation of the cells assessed by GPDH activity (Fig. 2). In contrast, preincubation of cells with the corresponding sense ODN failed to significantly affect the extent of differentiation (Fig. 2). Immunoblotting of JAK-2 immunoprecipitates with anti-phosphotyrosine antibodies after the antisense treatment confirmed an almost complete depletion of GH-activable JAK-2 from the cells (Fig. 2). These experiments indicate that JAK-2 is essential for GH-dependent differentiation of 3T3-F442A preadipocytes.

Role of MAP Kinases in the Differentiation of 3T3-F442A Cells—To determine the nature of the signals downstream of JAK-2 that are involved in the promotion of differentiation by GH, we first analyzed the role of the MAP kinase pathway. The
addition of GH to cells grown to confluence in GH-deficient medium induced a small activation of p42MAPK, as assessed by decreased electrophoretic mobility, that was transient and returned to basal levels after around 30 min (Fig. 3). A similar response was observed for activation of p44MAPK (data not shown). These data confirm that MAP kinases are activated during GH priming under these cell culture conditions. To test whether activation of these kinases is required for GH-dependent priming, we made use of the inhibitor PD 098059, which specifically inhibits the activation of mitogen-activated kinase and extracellular-regulated kinase kinase (MEK), the immediate upstream activator of p42MAPK and p44MAPK (22). Incubation of cells with PD 098059 during the 48-h priming period completely blocked the activation of MAP kinase by GH as assessed electrophoretic mobility (Fig. 4). Despite this blockade of MAP kinase activation, PD 098059 failed to inhibit the ability of GH to prime cells (Fig. 4). This was not due to a loss of PD 098059 activity during the 48-h priming period because the inhibitor retained its ability to block the activation of MAP kinase by GH after a 48-h preincubation (Fig. 4).

The above results suggested that the activation of MAP kinases by GH was not essential for the priming of cells by GH. However, because previous studies (14) with another preadipocyte cell line have shown a requirement of MAP kinases for adipogenic differentiation in the presence of serum, we tested whether they played any role in the differentiation of 3T3-F442A cells under serum-free conditions. GH induced an activation of p70s6k, as assessed by reduced electrophoretic mobility, that was sustained for at least 5 h (Fig. 6). To test the requirement for p42MAPK and p44MAPK in the differentiation process, cells were transfected with 5 μM antisense EAS1 or control ODNs and then subjected to differentiation using the defined differentiation medium (see “Experimental Procedures”). EAS1 caused a striking reduction in the extent of differentiation, as judged by lipid accumulation (data not shown), and a corresponding 98 ± 0.02% (p < 0.001; n = 3) reduction in GPDH activity (Fig. 5). Incubation with sense or scrambled ODNs did not significantly affect the extent of differentiation (Fig. 5). Therefore, the specific depletion of cellular MAP kinase blocks the transmission of differentiative stimuli in 3T3-F442A cells under serum-free conditions. The combined results of using antisense ODNs and the PD 098059 inhibitor strongly support the conclusion that activation of MAP kinases is not required for GH priming of confluent cells but that MAP kinases are required for the induction of terminal differentiation events by EGF, T3, and insulin.

The Role of the p70s6k Pathway in Differentiation—We next examined the requirement of the p70s6k signaling pathway for GH-dependent differentiation of 3T3-F442A cells under our defined serum-free conditions. GH induced an activation of p70s6k, as assessed by reduced electrophoretic mobility, that was sustained for at least 5 h (Fig. 6). To test the requirement for p70s6k activation, we used rapamycin, which blocks activation of p70s6k by all known agents (23). Rapamycin completely inhibited the activation of p70s6k by GH (Fig. 7) but failed to affect the subsequent terminal differentiation as assessed by GPDH activity (Fig. 7), indicating that p70s6k does not mediate the priming action of GH. Control experiments confirmed that rapamycin retained its ability to block p70s6k activation at the end of the 48-h incubation period (Fig. 7). Because an obliga-
tory role for the p70S6K pathway in the differentiation of 3T3-L1 cells in the presence of serum and insulin has been reported (24), we wished to establish whether it was similarly required for the overall process of differentiation under serum-free conditions. The activation of p70s6k by ETI was strong and was sustained above basal levels for 5–12 h (Fig. 6). The inclusion of rapamycin during terminal differentiation induced by ETI was found to inhibit adipogenic conversion by 58.7 ± 1.1% (p < 0.01; n = 3; Fig. 7). Thus, the p70S6K signaling pathway contributes significantly to the action of ETI during terminal differentiation but is not required for GH to prime cells for differentiation.

The Role of the STAT Pathway in Differentiation—The above data indicate that the ability of JAK-2 antisense ODNs to block GH-dependent differentiation is not due to the subsequent blocking of the MAP kinase or p70S6K pathways. In addition to activation of these pathways, JAK-2 activation leads to the phosphorylation of STAT transcription factors (see Ref. 10).

Treatment of cells with JAK-2 antisense ODNs but not sense or scrambled ODNs almost completely prevented GH-stimulated STAT-5 tyrosine phosphorylation (data not shown). To investigate whether STAT-5 was required for GH-dependent differentiation, we used an antisense ODN specifically to deplete STAT-5 from cells. Preincubation of 3T3-F442A preadipocytes with JAK-2 antisense ODNs but not sense or scrambled ODNs almost completely prevented GH-stimulated STAT-5 tyrosine phosphorylation (data not shown). To investigate whether STAT-5 was required for GH-dependent differentiation, we used an antisense ODN specifically to deplete STAT-5 from cells. Preincubation of 3T3-F442A preadipocytes with JAK-2 antisense ODNs but not sense or scrambled ODNs almost completely prevented GH-stimulated STAT-5 tyrosine phosphorylation (data not shown). To investigate whether STAT-5 was required for GH-dependent differentiation, we used an antisense ODN specifically to deplete STAT-5 from cells. 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with STAT-5 antisense ODNs caused a complete and specific depletion of cellular STAT-5a protein (Fig. 8), whereas the expression of STAT-1 and STAT-3 were unaffected (Fig. 8). These cells also contain STAT-5b, and the antisense treatment also resulted in a complete depletion of this isoform (data not shown). Treatment of cells with the corresponding sense or scrambled ODNs failed to affect the level of STAT-5a (Fig. 8) or STAT-5b (data not shown) protein expression. The requirement of STAT-5 proteins to support GH-dependent differentiation under serum-free conditions was then evaluated. Cells were transfected with 5 μM antisense STAT-5 or control ODNs before the induction of differentiation using the defined differentiation medium. Antisense STAT-5 ODNs caused a dramatic reduction (>99%) in the extent of differentiation, as judged GPDH activity (Fig. 8B). Incubation with sense or scrambled ODNs did not significantly affect the extent of differentiation. These results strongly implicate the JAK-STAT signaling pathway in transducing GH-dependent signals during the initiation of 3T3-F442A preadipocyte differentiation.

**DISCUSSION**

Although GH is well known to promote the differentiation of preadipocytes, the relevant intracellular signaling pathways involved have not been clearly identified. Because preadipocyte differentiation requires many extracellular factors individually acting at particular stages, it is difficult to dissect the contributions made by individual agents to the overall process. In this study, we have developed a serum-free system for the differentiation of 3T3-F442A preadipocytes in which the GH-dependent aspect of the process can be separated from the influence of other hormones. Several studies have indicated that GH acts at an early stage in differentiation by inducing an anti-mitogenic state and sensitizing cells to the actions of factors such as insulin, which then promote terminal differentiation (3–6).

Accordingly, we show that confluent cells can be primed with GH alone before the addition of other hormones which then induce terminal differentiation. Differentiation required treatment of cells with exogenous GH only if cells had been grown to confluence in medium containing serum depleted of GH. Thus, if cells are grown in normal calf serum, the endogenous GH present is sufficient to sensitize cells at confluence to the action of agents that induce terminal differentiation.

A wide body of evidence indicates that activation of the tyrosine kinase JAK-2 is critical and perhaps essential to GH action. Notably, mutant receptors, which do not interact with JAK-2, fail to activate MAP kinases and to induce gene activation (20, 21). Our JAK-2 antisense strategy now shows conclusively that JAK-2 is essential for GH-dependent differentiation of 3T3-F442A preadipocytes. Although tyrosine kinases of the JAK family are generally involved in signaling by cytokines, some evidence indicates that they also participate in signaling by other classes of growth factor. Of the other components of our serum-free medium essential for differentiation, both EGF (25) and insulin (26) have been shown to activate JAK tyrosine kinases. However, we have been unable to detect any EGF- or insulin-stimulated tyrosine phosphorylation of JAK-2 in 3T3-F442A preadipocytes or adipocytes. We therefore believe that JAK-2 is specifically involved in the GH-dependent priming stage of the differentiation process. To our knowledge, an absolute requirement for JAK kinases in the differentiation of preadipocytes or, indeed, of any other cells has not previously been reported. In the absence of synthetic JAK-2-specific inhibitors, the antisense strategy should prove useful in defining...

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2 N. G. Anderson, unpublished observations.
the role played by this kinase in other cellular processes.

Three major signaling pathways, which lie downstream of JAK-2 activation, are the MAP kinase pathway, the p70s6k pathway, and the STAT pathway (27–33). We have shown here that all three pathways are activated by GH during the priming of preadipocytes. However, although MAP kinases appear to be essential for the overall process of differentiation, blocking their activation with inhibitor PD 098059 during GH priming did not prevent subsequent differentiation. This indicates that MAP kinases are not involved in GH-dependent priming of the cells. Our data further show, for the first time, that MAP kinase activation is required specifically for terminal differentiation, which was induced in our study by the combination of EGF, T3, and insulin. The strong and sustained activation of MAP kinases induced by these factors in combination may result in nuclear translocation of the MAP kinases and, subsequently, the phosphorylation of key transcription factors. Further work will be required to identify the distal gene

FIG. 7. Effect of rapamycin on the priming and terminal differentiation of 3T3-F442A cells. A, confluent cells were treated as follows: no additions, lane 1; GH (2 nM, 20 min), lane 2; rapamycin (20 nM, 20 min) and then GH, lane 3; ETI, lane 4; rapamycin (20 min) and then ETI, lane 5; rapamycin (48 h) and then GH, lane 6. Lysates were then prepared and immunoblotted with anti-p70s6k antibodies as described under “Experimental Procedures.” B, cells were grown to confluence in the presence of GH-depleted calf serum. The medium was then removed, and cells were primed for 2 days in standard serum-free medium containing 2 nM GH. After this, the medium was replaced with medium containing EGF, T3, and insulin. Rapamycin (20 nM) was not added (NO ADDITION) or was added to cells during priming (GH PRIMING) or during terminal differentiation (TERMINAL DIFFN.). GPDH activities are the means ± S.E. for three observations and are expressed relative to activity measured in control (NO ADDITION) cultures (100%). Control GPDH activity was 213 ± 21 units/mg DNA (n = 3). * signifies that the value differs significantly from that for cells differentiated in the absence of rapamycin (NO ADDITION); p < 0.01.

FIG. 8. Effect of STAT-5 depletion on terminal differentiation. Confluent cells were treated with antisense STAT-5 (10 μM), scrambled oligonucleotides (10 μM), or sense oligonucleotides (10 μM) for 48 h with Lipofectin present for the first 8 h as described under “Experimental Procedures.” A, the levels of STAT-1, STAT-3, and STAT-5a were assessed by Western blotting with specific antisera as described under “Experimental Procedures.” Results are representative of three experiments. B, cells were grown to confluence in medium depleted of endogenous GH and then treated with antisense STAT-5 or control oligonucleotides. Cells were then transferred to the DDM in the continued presence of oligonucleotides. After 10 days, cells were harvested and processed for the assay of GPDH activity. Activity obtained in cultures differentiated with DDM alone was 192 ± 36 units/mg DNA (n = 3), and all values are expressed as a percentage of this activity. Results are the means ± S.E. for three observations.
targets for the MAP kinase pathway, which lead to terminal differentiation.

Our study also shows that, although GH induces a sustained activation of p70\(^{66k}\), this is not required for priming of the cells. Rapamycin, which specifically blocks p70\(^{66k}\) activation at the level of mammalian target of rapamycin (mTOR) (34), reduced the ability of ETI to support terminal differentiation of GH-primed cells but had no effect upon the priming action of GH. Therefore, the involvement of this pathway also appears to be restricted to terminal differentiation events.

Finally, this study has shown that GH induced the tyrosine phosphorylation of STAT-5a/5b during the priming of 3T3-F442A preadipocytes. This did not occur when differentiation was prevented by treatment of the cells with an antisense ODN to JAK-2. Moreover, specific depletion of STAT-5a/5b blocked activation of both p42/p44 MAP kinase and p70\(^{66k}\); these signaling pathways are not involved in the priming action of GH. By contrast, these signaling pathways are both necessary for terminal differentiation after GH priming. From our data, it appears that other JAK-2-dependent signaling pathways, including the activation of STAT-5a/5b, regulate this important biological action of GH.

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