Janus Kinase 2-dependent Activation of p38 Mitogen-activated Protein Kinase by Growth Hormone

RESULTANT TRANSCRIPTIONAL ACTIVATION OF ATF-2 AND CHOP, CYTOSKELETAL RE-ORGANIZATION AND MITOGENESIS*

(Received for publication, September 13, 1999, and in revised form, October 20, 1999)

Tao Zhu and Peter E. Lobe‡

From the Institute of Molecular and Cell Biology, National University of Singapore, 30 Medical Drive, Singapore 117609, Republic of Singapore

We demonstrate here that p38 mitogen-activated protein (MAP) kinase is activated in response to cellular stimulation by human GH (hGH) in Chinese hamster ovary cells stably transfected with GH receptor cDNA. This activation requires the proline-rich box 1 region of the GH receptor required for JAK2 association and is prevented by pretreatment of cells with the JAK2-specific inhibitor AG490. ATF-2 is both phosphorylated and transcriptionally activated by hGH, and its transcriptional activation also requires the proline-rich box 1 region of the GH receptor. Expression of wild type JAK2 can further enhance hGH-induced ATF-2, CHOP, and Elk-1-mediated transcriptional activation, whereas pre-treatment with AG490 is inhibitory. Use of either specific pharmacological inhibitors or transient transfection of cells with p38α MAP kinase cDNA or a dominant negative variant demonstrated that hGH-stimulated transcriptional activation of ATF-2 and CHOP, but not Elk-1, is regulated by p38 MAP kinase. Both the p38 MAP kinase and p44/42 MAP kinase are critical for hGH-stimulated mitogenesis, whereas only p38 MAP kinase is required for hGH-induced actin cytoskeletal re-organization. p38 MAP kinase is therefore an important regulator in coordinating the pleiotropic effects of GH.

* This work was supported by the National Science and Technology Board of Singapore (to P. E. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Institute of Molecular and Cell Biology, National University of Singapore, 30 Medical Drive, Singapore 117609, Republic of Singapore.

Growth hormone (GH),1 as the major regulator of postnatal body growth, possesses diverse and pleiotropic effects on the growth, differentiation, and metabolism of cells (1, 2). The GH receptor is a single membrane-spanning glycoprotein in the cytokine receptor superfamily (3) that consists of a ligand-binding external domain and a 350-amino acid residue cytoplasmic domain required for intracellular signal transduction (1, 2). GH receptor signaling involves ligand-induced receptor homodimerization and activation of the tyrosine kinase JAK2 by association with the GH receptor (1, 2, 4). The proline-rich Box1 motif of the receptor has been identified as the site of association with JAK2. Multiple effector molecules downstream of JAK2-mediating GH signal transduction have now been identified (5–11). We have also recently demonstrated that GH stimulates the formation of a multiprotein signaling complex centered around p125FAK (12) and p130Cas-CrkII (13) leading to JNK/SAPK activation.

The mitogen-activated protein kinase (MAPK) superfamily, encompassing p44/42 MAP kinase, c-Jun amino-terminal kinases (JNK), and p38 MAP kinase, are proline-directed serine-threonine protein kinases that have important functions as mediators of cellular responses to a variety of extracellular stimuli (14, 15). p44/42 MAP kinase has been implicated in mitogenic growth in a variety of cell contexts, and its activation is via a well known sequential cascade involving SHC, Grb2, son-of-sevenless, Ras, Raf, and MAP/extracellular signal-regulated kinase (MEK) (14, 15). The p38 MAP kinase genes have been shown to be activated by a series of cytokines, growth factors, and autonomic neurotransmitters (16–19) in addition to the stress and pro-inflammatory signals (20–22). Activation of p38 MAP kinase involves phosphorylation on threonine and tyrosine residues present in a TGY amino acid motif (23, 24), resulting in increased enzyme activity (25, 26). At least four isoforms of p38 MAP kinase have been described (20–22, 26–30). Experiments with dominant-negative or active mutant proteins have demonstrated that p38 MAP kinase lies downstream of Ras, Cdc42, GCK, PKA, TAK1, ASK1, TPL1, and RAFTK/PYK2 (31–39) and is directly activated by three dual-specificity MAPK kinases, MKK3, MKK4, and MKK6 (36, 40–43). Some of the substrates for p38 MAP kinase that may be physiologically relevant have been identified. These include the transcription factors ATF-2 (23, 42), CHOP (44), Elk-1 (45, 46), CREB, ATF-1 (47, 48), Sap1a (45, 46), MEF2C, and MEF-2A (49–51), which can be phosphorylated and transcriptionally activated by p38 MAP kinase. p38 MAP kinase also activates the following downstream protein kinases: MAPKAP kinase-2, MAPKAP kinase-3, and p38-regulated/activated protein kinase (22, 52, 53).

We and others (54–56) have previously demonstrated GH activation of both p44/42 MAP kinase and c-Jun amino-terminal kinase (JNK/SAPK) (13). We demonstrate here that hGH transiently phosphorylates and activates p38 MAP kinase in CHO cell lines stably transfected with rat GH receptor cDNA and that the activation of the p38 MAP kinase pathway by hGH is JAK2-dependent. Furthermore, both ATF-2 and CHOP (GADD153) are transcriptionally activated by hGH in a JAK2-dependent manner, and p38 MAP kinase is required for both cytoskeletal re-organization and cell proliferation stimulated by hGH. The p38 MAP kinase pathway is therefore an important regulator of the pleiotropic effects of GH.
**EXPERIMENTAL PROCEDURES**

Materials—Recombinant human growth hormone (hGH) was a generous gift of Novo-Nordisk (Singapore). The JAK2 inhibitor tyrphostin AG490, MEKi inhibitor PD98059, and the p38 MAP kinase inhibitor SB203580 were purchased from Calbiochem. Protein A/G plus agarose was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The enhanced chemiluminescence (ECL) kit was purchased from Amersham Pharmacia Biotech. The immortalized p38α MAP kinase cell line (Thr-180/Tyr-182) monoclonal antibody, immobilized phospho-p44/42 MAP kinase (Thr-202/Tyr-204) monoclonal antibody, phosho-p38 MAP kinase (Thr-180/Tyr-182) polyclonal antibody, phospho-ATF-2 (Thr-71) polyclonal antibody, ATF-2 polyclonal antibody, phospho-Elk-1 (Ser-383) polyclonal antibody, Elk-1 polyclonal antibody, and Elk-1 fusion protein were purchased from New England Biolabs (Beverly, MA). The glutathione S-transferase fusion protein of the amino-terminal portion of Elk-1 were purchased from Stratagene (La Jolla, CA). pFC2-dbd plasmid was a generous gift of Dr. Nils Billestrup.

Cell Culture and Treatment—CHO Cell Lines Stably Transfected with GH Receptor cDNA—Rat GH receptor cDNAs were cloned into an expression plasmid containing an SV40 enhancer and a human metallothionein IIA promoter. The cDNAs were transfected into CHO-K1 cells using Lipofectin together with the pIJβ-1 plasmid, which contains a neomycin resistance gene fused to the thymidine kinase promoter. Stable integrants were selected using 1000 μg/ml G418. The complete rat GH receptor cDNA coding for amino acids 1–639 was expressed in CHO40–638 or CHOα-638 cells and will be referred to as CHO-GHR1–638 (54). The construction of GH receptor cDNA expression plasmids containing a deletion of box 1 (Δ297–311) and the individual substitution of proline residues 300, 301, 303, and 305 in box 1 for alanine has been described previously (59). These cDNAs were stably transfected into CHO-K1 cells; the Δ297–311 mutation was expressed in CHO-GHR1–Δ297–311 cells, P300Δ301,503A,505A was expressed in CHO-GHR1–Δ301 in CHO-GHR1–Δ301 cells, and P300Δ301,303A,305A was expressed in CHO-GHR1–Δ301,303A,305A cells (59). The level of receptor expression for the individual cell clones is comparable between clones and has been described previously (54, 59).

Cell Culture and Treatment—CHO-GHR1–Δ301, CHO-GHR1–Δ301,303A,305A cells were maintained in Ham’s F-12 medium (F-12) plus 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO2 and 95% air at 37 °C as described previously (59). Prior to treatment, cells were deprived of serum for 12–16 h in Ham’s F-12 medium. Unless otherwise indicated, the final concentration of the MEK1 inhibitor PD98059 was 30 μM, p38 MAP kinase inhibitor SB203580 was 10 μM, and hGH was 50 nM. This concentration of GH is within the physiological range for circulating rodent GH (60, 61).

Immunoblotting—Immunoblotting for p38 MAP kinase, Elk-1, phospho-Elk-1, ATF-2, and phospho-ATF-2 were carried out as described previously (12, 13). After preincubation with inhibitors for respective time and/or incubation with hGH for the appropriate duration, the cells were washed twice with ice-cold PBS, and cells were lysed at 4 °C in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.4, 1% Triton-100, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mM sodium orthovanadate, 0.5% Nonidet P-40, 10 μg/ml aprotinin, and leupeptin) for 30 min with regular vortexes. Cell lysates were centrifuged at 14,000 × g for 15 min, and the resulting supernatants were collected, and protein concentration was determined. Cell lysates dissolved in 1× SDS-polyacrylamide gel electrophoresis sample buffer containing 25 mM Tris-HCl, pH 6.8, 1% SDS, 1% mercaptoethanol, and bromphenol blue, boiled for 10 min, and centrifuged at 14,000 × g for 2 min were analyzed on 10% SDS-

polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat dry milk in phosphate-buffered saline with 0.1% Tween 20 (PBST) for 1 h at 22 °C. The blots were then treated with the primary antibody in PBST containing 1% non-fat dry milk at 4 °C overnight. After three washes with PBST, immunoblots were detected by Enhanced chemiluminescence (ECL) as described above (50). Membranes were then stripped by incubation for 30 min at 50 °C in a solution containing 62.5 mM Tris-HCl, pH 6.7, 2% SDS, and 0.7% mercaptoethanol. Blots were then washed for 30 min with several changes of PBST at room temperature. Efficacy of stripping was determined by re-exposure of the membranes to ECL. Thereafter, blots were reblocked and re-labeled as described above.

**Kinase Assays—p38 MAP kinase assays were performed using New England Biolabs assay kit specific for p38α and p38β MAP kinase according to the manufacturer’s instructions. In brief, CHO-GHR1–Δ301 cells were serum-deprived for 16 h, treated with 50 nM hGH, and the cells lysed at 4 °C in 1 ml of lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM glycerol phosphate, 1 mM Na3VO4, 0.1% phenylmethylsulfonyl fluoride, 1 mg/ml leupeptin) per sample. The lysates were centrifuged at 15,000 × g for 15 min at 4 °C. The supernatant containing 0.5 μg of protein per sample was incubated overnight at 4 °C with 20 μl of immobilized phospho-p38 MAP kinase (Thr-180/Tyr-182) monoclonal antibody in a final volume of 500 μl in 1× lysis buffer. Phosphorylation of p38 MAP kinases was selectively detected with 0.1% phenylmethylsulfonyl fluoride and with 500 μg of kinase buffer (25 mM Tris-HCl, pH 7.5, 5 mM glycerol phosphate, 2 mM diithiothreitol, 0.1 mM Na3VO4, 10 mM MgCl2, 3 μg of recombinant glutathione S-transferase-ATF2 protein containing 1–109 amino-terminal amino acids of ATF-2 was added to each sample. The kinase reactions were carried out in the presence of 100 μM ATP at 30 °C for 30 min. Phosphorylation of ATP-2 at Thr-71 was determined by Western blot using phospho-ATF-2 (Thr-71) antibody (1000). p44/42 MAP kinase assays were also performed using New England Biolabs assay kit according to the manufacturer’s instructions. In brief, cells were serum-deprived for 16 h, treated with 50 nM hGH, and lysed at 4 °C in 1 ml of lysis buffer (as described above) per sample. The lysates were centrifuged at 15,000 × g for 15 min at 4 °C. The supernatant containing 200 μg of protein per sample was incubated overnight at 4 °C with 15 μl of immobilized phospho-specific p44/42 MAP kinase (Thr-202/Tyr-204) monoclonal antibody in a final volume of 500 μl in 1× lysis buffer. The pellets were washed twice with 500 μl of lysis buffer containing 0.1% phenylmethylsulfonyl fluoride and washed twice with 500 μg of kinase buffer (25 mM Tris-HCl, pH 7.5, 5 mM glycerol phosphate, 2 mM dithiothreitol, 0.1 mM Na3VO4, 10 mM MgCl2). The kinase reactions were performed in the presence of 2 μl of Elk-1 fusion protein and 200 μl ATP at 30 °C for 30 min. Elk-1 phosphorylation was selectively detected by western immunoblotting using a chemiluminescent detection system and a specific phospho-Elk-1 (Ser-383) antibody (1000).

**Transfection Procedure and Luciferase Assays—**CHO-GHR1–Δ301 cells were cultured to 60–80% confluence for transfection experiments in 6-well plates (1 μg of recombinant plasmid DNA and 2 ml of lipofectin per well) together with 20 ng of the respective fusion trans-activator plasmid (pFA-ATF-2, pFA-ChOP, pFA-Elk-1, or pFC2-dbd). For transient transfection of MAP kinases, either 1.5 μg of pcDNA3-p38α MAPK, dominant negative pcDNA3-p38α MAPK, or pcDNA3-p42 MAPK were transfected in each well. Control empty vector was used to normalize the amount of plasmids in each well. For each well, 4 μl of DOTAP for each μg of DNA was used as per the manufacturer’s instructions. DNA and the DOTAP reagents were diluted separately in 100 μl of serum-free medium mixed and incubated at room temperature for 30 min. DNA-lipid complex was diluted to a final volume of 6 ml (for triplicate samples) with serum-free medium. Cells in each well were rinsed once with 2 ml of serum-free medium, and 2 ml of diluted DNA-lipid complex was overlaid in each well and incubated for 6 h. After incubation, complete Ham’s F-12 medium containing 2% FBS was added to each well so as to incubate the cells in 0.5% serum for 12–16 h. Cells were pretreated with 10 μM SB203580 or 30 μM PD98059 or with the equivalent amount of solvent (MeSO) as a control for 45 min prior to stimulation with hGH. 50 nM hGH was added for an additional 5–7 h. The cells were rinsed with 3 ml of serum-free medium mixed and incubated at 14,000 rpm for 15 min. The supernatant was used for the assay of luciferase and β-galactosidase activity. The luciferase activities were normalized on the basis of protein content as well as on the β-galactosidase activity of pCMVβ vector. The β-galactosidase assay was performed with 20 μl of precleared cell lysate according to a
standard protocol (62). Mean and standard deviations of at least three independent experiments are shown in the figures.

Visualization of Filamentous Actin and Confocal Laser Scanning Microscopy—Cells were fixed in ice-cold 4% paraformaldehyde, washed with PBS, permeabilized for 5 min with 0.1% Triton X-100, blocked in 2% bovine serum albumin, and incubated with phalloidin-TRITC (0.2 mg/ml) after the indicated treatments. Excess phalloidin-TRITC was removed by extensive washing with PBS. Labeled cells were visualized with a Carl Zeiss Axiosplan microscope equipped with epifluorescence optics and a Bio-Rad MRC1024 confocal laser system. Images were converted to the tagged information file format and processed with the Macintosh Photoshop program.

Cell Proliferation Assays—Cell proliferation was assayed by measuring incorporation of [3H]thymidine during DNA synthesis (63). Subconfluent CHO-GHR<sub>1–638</sub> cell monolayers in 24-well plates were grown to quiescence in serum-free Ham’s F-12 medium at 37 °C for 16 h. Cells were then incubated for 24 h in Ham’s F-12 medium with hGH to a final concentration as indicated in the figure legends. Each cell line was plated in triplicate for each treatment. In case of pretreatment of cells with chemical inhibitors, cells were pretreated with 10 μM SB203580 or 30 μM PD98059 or with the equivalent amount of solvent (Me<sub>2</sub>SO) as a control for 45 min. [3H]Thymidine (1 μCi per well, 1 Ci = 37 GBq, Amersham Pharmacia Biotech) was added, and the cells were incubated at 37 °C for a further 8 h. Cells were rinsed twice with ice-cold PBS incubated with 1 ml of ice-cold 5% trichloroacetic acid for 30 min at 4 °C and 0.5 ml of 0.5 N NaOH. 0.5% SDS was added subsequently at room temperature. Solubilized samples were subjected to liquid scintillation counting in a scintillation counter.

Statistics and Presentation of Data—All experiments were repeated at least three times. Figures presented for Western blot analyses are representative of multiple experiments. The text under “Results” summarizes the results from multiple Western blot analyses. Consequently, the text under “Results” (e.g. description of the time course of phosphorylation and dephosphorylation) may not exactly correspond to the actual figure presented. All numerical data are expressed as mean ± S.D. Data were analyzed using the two-tailed t test or analysis of variance.

RESULTS

Time- and Dose-dependent Activation of p38 MAP Kinase by Cellular Stimulation with hGH—We and others (13, 54–56) have previously shown that GH activates both p42/44 MAP kinase and JNK. In order to determine whether cellular stimulation with hGH also results in the activation of p38 MAP kinase, we first examined whether hGH stimulation of cells resulted in the phosphorylation of p38 MAP kinase. Dual phosphorylation of p38 MAP kinase on Thr-180 and Tyr-182 is required for its activation (14, 25, 64). We therefore treated serum-deprived CHO-GHR<sub>1–638</sub> cells for 0–60 min with 50 nm hGH and prepared cell extracts for Western blot analysis. Phosphorylated p38 MAP kinase was detected by use of a phospho-p38 MAP kinase (Thr-180/Tyr-182) antibody that detects p38 MAP kinase after dual phosphorylation at Thr-180 and Tyr-182. hGH stimulation of CHO-GHR<sub>1–638</sub> cells resulted in the phosphorylation of p38 MAP kinase. Dual phosphorylation of p38 MAP kinase in Thr-180 and Tyr-182 is required for its activation (14, 25, 64). We therefore treated serum-deprived CHO-GHR<sub>1–638</sub> cells for 0–60 min with 50 nm hGH and prepared cell extracts for Western blot analysis. Phosphorylated p38 MAP kinase was detected by use of a phospho-p38 MAP kinase (Thr-180/Tyr-182) antibody that detects p38 MAP kinase after dual phosphorylation at Thr-180 and Tyr-182. hGH stimulation of CHO-GHR<sub>1–638</sub> cells resulted in increased p38 MAP kinase phosphorylation detectable 5 min after stimulation. Maximal hGH-stimulated phosphorylation of p38 MAP kinase was observed at 15 min and followed by a subsequent decline in phosphorylation state to 60 min (Fig. 1A). Equal loading of samples was verified by stripping of the membrane and subsequent reblotting with a p38 MAPK antibody (Fig. 1B). We also examined whether the hGH-stimulated phosphorylation of p38 resulted in enhanced p38 MAP kinase activity using ATF-2 as an in vitro substrate (26, 41). We therefore treated serum-deprived cells for 0–60 min with 50 nm hGH and immunoprecipitated dual-phosphorylated p38 MAP kinase from cell extracts with immobilized phospho-p38 MAP kinase (Thr-180/Tyr-182) monoclonal antibody and measured p38 MAP kinase activity by using ATP-2 as the substrate. Phosphorylation of ATP-2 by the immunoprecipitated p38 MAP kinase was detected by immunoblotting with an antibody that detects ATP-2 when phosphorylated on threonine residue 71 (Thr-71). Thus the appearance of phosphorylation of p38 MAP kinase observed in hGH-stimulated cell extracts corresponds temporally to the increase in p38 MAP kinase activity in hGH-stimulated cell extracts. We also examined the dose dependence of the hGH stimulation of p38 MAP kinase phosphorylation and activity. We therefore stimulated CHO-GHR<sub>1–638</sub> cells with 0, 0.005, 0.05, 0.5, 5, and 50 nm hGH for 15 min and prepared cell extracts. Determination of p38 MAP kinase phosphorylation and p38 MAP kinase activity was performed as described above. The maximal phosphorylation (Fig. 1D) and activation (Fig. 1F) of p38 MAP kinase was achieved at a concentration of 50 nm hGH in CHO-GHR<sub>1–638</sub> cells. Again, equal loading of samples was verified by stripping of the membrane and subsequent reblotting with a p38 MAPK antibody (Fig. 1E). Therefore, 50 nm hGH was used for all subsequent experiments.

Activation of p38 MAP Kinase by hGH Is JAK2-dependent—The activation of JAK2 upon cellular stimulation with GH is thought to be required for subsequent signal transduction events (4, 5, 12, 65). To examine the potential role of JAK2 in the activation of p38 MAP kinase by hGH, we utilized well characterized CHO cell clones stably expressing the wild type receptor (CHO-GHR<sub>1–638</sub>), a receptor mutation in which the proline-rich box 1 region had been deleted (CHO-GHR<sub>1–638</sub>Δ-
A receptor mutation in which the individual proline residues of box 1 had been converted to alanine (CHO-GHR1–638P300,301,303,305A) (Fig. 2A). The proline rich box 1 region of the GH receptor is required for the association of JAK2 with the receptor and its subsequent activation after receptor dimerization (59). We therefore stimulated the respective serum-deprived cell line with 50 nM hGH for 15 min and prepared cell extracts for determination of p38 MAP kinase activity. hGH stimulation of CHO-GHR1–638 cells resulted in activation of p38 MAP kinase, whereas no hGH-dependent activation of p38 MAP kinase activity was observed in either the CHO-GHR1–638D297–311 or CHO-GHR1–638P300,301,303,305A cell lines (Fig. 2B). For control purposes we also treated CHO-GHR1–638, CHO-GHR1–638D297–311, and CHO-GHR1–638P300,301,303,305A cell lines with sorbitol (41, 66) and demonstrated equipotent activation of p38 MAP kinase in all three cell lines. Thus it is apparent that the hGH activation of p38 MAP kinase requires the proline-rich box 1 region of the GH receptor indicative that p38 MAP kinase is activated in a JAK2-dependent manner.

Tyrphostin AG490, a reportedly specific inhibitor of JAK2 tyrosine kinase activity (67–69), was also utilized to verify that hGH stimulation of p38 MAP kinase activity was JAK2-dependent. CHO-GHR1–638 cells were therefore pretreated with 100 μM AG490 or vehicle (Me2SO) for 16 h, followed by stimulation with 50 nM hGH for 0 or 15 min, cell extracts prepared, and p38 MAP kinase activity determined (C). CHO-GHR1–638 cell (preincubated 6 AG490), CHO-GHR1–638D297–311 cells, and CHO-GHR1–638P300,301,303,305A cells were treated with sorbitol for 5 min, cell extracts prepared, and p38 MAP kinase activity determined (D). The data presented are representative of at least three separate experiments.

Activation of p38 MAP kinase by hGH in CHO cells stably transfected with wild type GH receptor or Box1-deficient GH receptor cDNAs. A, schematic diagram of the GH receptor and the various GH receptor mutations/deletions used. The wild type receptor has the extracellular, transmembrane, and intracellular regions indicated. CHO-GHR1–638D297–311 has the proline-rich region (Box1) deleted, CHO-GHR1–638P300,301,303,305A has the individual proline residues in box 1 mutated to alanine. CHO-GHR1–638 cells and CHO cells expressing the GH receptor box 1 mutations or deletions were stimulated with 50 nM hGH for 15 min, cell extracts prepared, and p38 MAP kinase activity determined (B). CHO-GHR1–638 cells were preincubated with 100 μM tyrphostin AG490 or vehicle Me2SO for 16 h at 37 °C prior to treatment with 50 nM hGH for 0 or 15 min, cell extracts prepared, and p38 MAP kinase activity determined (C). CHO-GHR1–638 cell (preincubated 6 AG490), CHO-GHR1–638D297–311 cells, and CHO-GHR1–638P300,301,303,305A cells were treated with sorbitol for 5 min, cell extracts prepared, and p38 MAP kinase activity determined (D). The data presented are representative of at least three separate experiments.

297–311), and a receptor mutation in which the individual proline residues of box 1 had been converted to alanine (CHO-GHR1–638D297–311 or CHO-GHR1–638P300,301,303,305A) (Fig. 2A). The proline rich box 1 region of the GH receptor is required for the association of JAK2 with the receptor and its subsequent activation after receptor dimerization (59). We therefore stimulated the respective serum-deprived cell line with 50 nM hGH for 15 min and prepared cell extracts for determination of p38 MAP kinase activity. hGH stimulation of CHO-GHR1–638 cells resulted in activation of p38 MAP kinase, whereas no hGH-dependent activation of p38 MAP kinase activity was observed in either the CHO-GHR1–638D297–311 or CHO-GHR1–638P300,301,303,305A cell lines (Fig. 2B). For control purposes we also treated CHO-GHR1–638, CHO-GHR1–638D297–311, and CHO-GHR1–638P300,301,303,305A cell lines with sorbitol (41, 66) and demonstrated equipotent activation of p38 MAP kinase in all three cell lines. Thus it is apparent that the hGH activation of p38 MAP kinase requires the proline-rich box 1 region of the GH receptor indicative that p38 MAP kinase is activated in a JAK2-dependent manner.
GH Activation of p38 MAP Kinase

Phosphorylation of the ATF-2 by hGH Is JAK2-dependent—We also examined the requirement of JAK2 for hGH-stimulated phosphorylation of ATF-2. We utilized the same well characterized CHO cell clones stably expressing either the wild type receptor (CHO-GHR1–638), a receptor mutation in which the proline-rich box 1 region had been deleted (CHO-GHR1–638P300,301,303,305A), or a receptor mutation in which the proline-rich box 1 region had been deleted (CHO-GHR1–638P300,301,303,305A) as described above. Each serum-deprived cell line was stimulated with 50 nM hGH for 15 min and prepared cell extracts. The maximal phosphorylation of ATF-2 was achieved at a concentration of 5–50 nM hGH for 30 min. The samples containing equal amounts of proteins were electrophoresed and immunoblotted with the antibodies specific for ATF-2 phosphorylated at Thr-71 (A) and total ATF-2 (B). CHO-GHR1–638 cells were preincubated with 100 μM tyrphostin AG490 or vehicle MeSO for 16 h at 37 °C prior to treatment with 50 nM hGH. The samples containing equal amounts of proteins were electrophoresed and immunoblotted with the antibodies specific for ATF-2 phosphorylated at Thr-71 (C) and total ATF-2 (D). The data presented are representative of at least three separate experiments.

hGH Stimulates the Transcriptional Activation of ATF-2, CHOP, and Elk-1—To determine if hGH-stimulated phosphorylation of ATF-2 resulted in ATF-2-dependent transcription, we utilized a trans-activation reporter assay specific for ATF-2. We therefore transiently transfected CHO-GHR1–638 cells with the fusion trans-activator plasmid pFA-ATF-2 consisting of the DNA binding domain of GAL4 (residue 1–147) and the trans-activation domain of ATF-2 together with the fusion trans-activator plasmids pFA-ATF-2 consisting of the DNA binding domain of GAL4 (residue 1–147) and the trans-activation domain of ATF-2, together with luciferase reporter plasmid and pCMVβ vector, respectively. The luciferase activities were measured and normalized on the basis of protein content as well as on the β-galactosidase activity of pCMVβ vector. We demonstrated an hGH-dependent transcriptional activation of ATF-2 (Fig. 5A) in CHO-GHR1–638 cells. hGH failed to stimulate ATF-2-mediated reporter expression in cells transfected with a plasmid encoding the GAL4 DNA binding domain (residue 1–147) lacking an activation domain, indicative that the ATF-2 transcriptional activation domain is required for hGH-stimulated reporter expression. Since transcriptional activation of CHOP has also been shown to be p38 MAP kinase-dependent, we utilized a trans-activation reporter assay specific for CHOP to determine if hGH can also stimulate CHOP-dependent transcription. We demonstrated a hGH-dependent transcriptional activation of CHOP (Fig. 5B) in CHO-GHR1–638 cells. hGH failed to stimulate CHOP-mediated reporter expression in cells transfected with a plasmid encoding the GAL4 DNA binding domain (residue 1–147) lacking an activation domain, indicating that the CHOP transcriptional activation domain is required for hGH-stimulated reporter expression.

We have also incorporated an Elk-1 reporter assay for control purposes (Fig. 5C), since it was previously shown that GH-stimulated transcriptional activation of Elk-1 was p44/42 MAP kinase-dependent (70, 71). We demonstrated a hGH-dependent transcriptional activation of Elk-1 in CHO-GHR1–638 cells. hGH failed to stimulate Elk-1-mediated reporter expression in cells transfected with a plasmid encoding the GAL4 DNA binding domain (residue 1–147) lacking an activation domain, indicating that the Elk-1 transcriptional activation domain is required for hGH-stimulated reporter expression.

hGH-stimulated ATF-2, CHOP, and Elk-1 Transcriptional Activation Is JAK2-dependent—We have demonstrated above that the activation of p38 MAP kinase by hGH is JAK2-dependent and therefore reasoned that hGH-stimulated transcriptional activation of ATF-2 and CHOP should also be JAK2-dependent. hGH stimulation of Elk-1 has previously been demonstrated to be JAK2-dependent (73). We first examined the hGH-dependent transcriptional activation of ATF-2, CHOP, and Elk-1 in CHO cell clones stably expressing either the wild type receptor (CHO-GHR1–638), a receptor mutation in which the proline-rich box 1 region had been deleted (CHO-GHR1–638P300,301,303,305A), or a receptor mutation in which the proline-rich box 1 region had been deleted (CHO-GHR1–638P300,301,303,305A) as described above. Neither the CHO-GHR1–638A297–311 nor CHO-GHR1–638P300,301,303,305A cell lines responded to hGH with transcriptional activation of ATF-2, CHOP, or Elk-1 in contrast to CHO-GHR1–638 cells that responded with the expected magnitude of transcriptional
activation (Fig. 6A). We also demonstrated that transient transfection of a JAK2 expression plasmid enhanced the hGH-dependent transcriptional activation of ATF-2, CHOP, and Elk-1. Furthermore, pretreatment of the cells with 100 μM tyrphostin AG490 prevented both the hGH-dependent transcriptional activation of ATF-2, CHOP, and Elk-1 and the enhancement of hGH-dependent transcriptional activation observed upon transient transfection of the JAK2 ex-
Lack of Cross-inhibition between the MEK1-specific Inhibitor and the p38 MAP Kinase-specific Inhibitor—

PD98059 selectively blocks the activity of MEK1 thereby inhibiting the activation of the p44/42 MAP kinase and the subsequent phosphorylation of p44/42 MAP kinase substrates both in vitro and in vivo (73–75). SB203580 is a pyridinyl imidazole derivative that is a highly specific inhibitor of p38 MAP kinase activity (26, 76, 77). We utilized these pharmacologic agents to determine which pathway was utilized by hGH to stimulate the transcriptional activation of ATF-2, CHOP, and Elk-1 and therefore needed to establish the absence of cross-inhibition of the respective pathways. CHO-GHR1–638 cells were pretreated with either 30 µM PD98059 or 10 µM SB203580 prior to hGH stimulation, and the activity of both MAP kinases was determined.

30 µM PD98059 did not inhibit the hGH-stimulated activation of p38 MAP kinase (Fig. 7A), and 10 µM SB203580 did not inhibit the hGH-stimulated activation of p42/44 MAP kinase (Fig. 7B). Both of the inhibitors at the same concentration specified above afforded inhibition of the desired respective pathway (Fig. 7A and B). Thus, PD98059 and SB203580 were suitable for delineation of the MAP kinase pathway required for hGH-dependent activation of ATF-2, CHOP, and Elk-1 in the CHO-GHR1–638 cell line.

Inhibition of p38 MAP Kinase Attenuates hGH-stimulated ATF-2 and CHOP but Not Elk-1-mediated Transcriptional Activation—To investigate the possible role of p44/42 MAP kinase and p38 MAP kinase in hGH-induced ATF-2- and CHOP-mediated transcriptional activation, CHO-GHR1–638 cells transfected with the fusion transactivator plasmid pFA-ATF2 or pFA-CHOP, and the luciferase reporter plasmids were treated with PD98059, SB203580, or Me2SO prior to hGH stimulation. Human GH-stimulated ATF-2-mediated transcriptional activation in CHO-GHR1–638 cells was significantly abrogated by the pretreatment with SB203580 but was unaffected by pretreatment with PD98059, indicating that the hGH-stimulated ATF-2-dependent gene expression requires p38 MAP kinase activation but not MEK1 and subsequent p44/42 MAP kinase activation.

**Fig. 7.** Inhibition of p38 MAP kinase attenuates hGH-stimulated ATF-2 and CHOP but not Elk-1-mediated transcriptional activation. CHO-GHR1–638 cells were preincubated with the MEK1 inhibitor PD98059 (30 µM) or the p38 MAP kinase inhibitor SB203580 (10 µM) for 45 min prior to treatment with 50 nM hGH for 15 min. Cell extracts were prepared, and p38 MAP kinase activity (A) and p44/42 MAP kinase activity (B) were determined as described under “Experimental Procedures.” CHO-GHR1–638 cells were co-transfected with the following: 1) β-galactosidase expression vector pCMVβ; 2) reporter plasmid pFR-Luc; 3) fusion trans-activator plasmid either pFA-ATF2 (C), pFA-CHOP (D), or pFA2-Elk1 (E). CHO-GHR1–638 cells were preincubated with MEK1 inhibitor PD98059 (30 µM), p38 MAP kinase inhibitor SB203580 (10 µM), or vehicle (Me2SO) as indicated for 45 min prior to treatment with 50 nM hGH for 6–8 h. The relative luciferase activities presented were normalized by protein concentrations as well as β-galactosidase activity (mean ± S.D., n = 3). Cells were treated with vehicle (solid bars) or hGH (solid bars) as above. The data presented are representative of at least three separate experiments. Bars represent mean ± S.D. *, p < 0.01.
separate experiments.

The results demonstrate that p38 MAP kinase plays a role in hGH-dependent transcriptional activation. CHO-GHR1–638 cells transfected with pFA-ATF-2, pFA-CHOP, or pFA2-Elk1, and the indicated p38a MAP kinase, p38a MAP kinase dominant negative, or the p42 MAP kinase (ERK2) expression plasmids. CHO-GHR1–638 cells were incubated with 50 nM hGH for 6–8 h, and cell extracts were prepared. The relative luciferase activities were normalized by protein concentrations as well as β-galactosidase activity (mean ± S.D., n = 3). Cells were treated with vehicle (hatched bars) or hGH (solid bars) as above. Luciferase activity in cell lysates is normalized to the activity in untreated control cells (control = 1). The data presented are representative of at least three separate experiments. Bars represent mean ± S.D. *, p < 0.01.

Expression of p38a MAP Kinase Enhances hGH-induced ATF-2- and CHOP-mediated transcriptional activation—To verify further that hGH-stimulated ATF-2- and CHOP-mediated transcriptional activation are downstream of p38 MAP kinase and are independent of p44/42 MAP kinase activation. In contrast, both p44/42 MAP kinase and p38 MAP kinase have shown to be upstream of Elk-1 (78). To determine whether inhibition of the p44/42 MAP kinase or the p38 MAP kinase activity affected the ability of Elk-1 to mediate transcriptional activation in response to hGH, CHO-GHR1–638 cells transfected with pFA-Elk-1 fusion transactivator plasmid and luciferase reporter plasmid were treated with 30 μM PD98059, 10 μM SB203580, or Me2SO prior to hGH stimulation. Human GH-stimulated Elk-1-mediated transcriptional activation in CHO-GHR1–638 cells was largely abrogated by pretreatment with PD98059 but unaffected by pretreatment with SB203580, indicating that hGH-stimulated Elk-1-dependent gene expression requires MEK1 activation but not p38 MAP kinase activation (Fig. 7E).

Expression of p38α MAP Kinase Enhances hGH-induced ATF-2- and CHOP-mediated but Not Elk-1-mediated transcriptional activation—To verify further that hGH-stimulated ATF-2 and CHOP-mediated transcriptional activation were p38 MAP kinase-dependent, we transiently transfected either wild type p38α MAP kinase, dominant negative p38α MAP kinase, or p42 MAP kinase expression plasmids with the respective fusion trans-activator plasmids (pFA-ATF2, pFA-CHOP, and pFA2-Elk1) and the luciferase reporter plasmid and examined hGH-stimulated ATF-2, CHOP, and Elk-1-mediated transcription. As observed in Fig. 8, transfection of wild type p38α MAP kinase enhanced both hGH-induced ATF-2- and CHOP- but not Elk-1-mediated transcriptional activation. Transfection of the dominant negative p38α MAP kinase abolished both hGH-induced ATF-2- and CHOP-mediated but not Elk-1-mediated transcription. Transfection of p42 MAP kinase did not alter hGH-induced ATF-2- or CHOP-mediated transcriptional activation, although hGH-induced Elk-1-mediated transcriptional activation was remarkably enhanced upon cotransfection of p42 MAP kinase. These data suggest that activation of p38 MAP kinase is required for hGH-dependent ATF-2- and CHOP-mediated but not Elk-1-mediated transcriptional activation in CHO-GHR1–638 cells.

hGH-induced Actin Cytoskeletal Re-organization Is p38 MAP Kinase-dependent—hGH has previously been demonstrated to stimulate re-organization of the actin cytoskeleton including stress fiber breakdown and the formation of membrane ruffles (79). Since p38 MAP kinase has been implicated to be required for actin cytoskeletal reorganization stimulated by a variety of stimuli (18, 80, 81), we therefore examined whether pretreatment of cells with SB203580 affected hGH-induced actin cytoskeletal re-organization. CHO-GHR1–638 cells were grown on coverslips to 50% confluence and pretreated with PD98059 or SB203580 or the equivalent volume of Me2SO for 45 min followed by stimulation with 50 nM hGH for 5 min. In vehicle-treated cells, hGH initially stimulated the depolymerization of actin stress fibers with maximal depolymerization of stress fibers observed 5 min after addition of hGH (Fig. 9, A and B). Pretreatment of cells with SB203580 prevented the ability of hGH to stimulate stress fiber breakdown in CHO-GHR1–638 cells (Fig. 9D). In contrast, CHO-GHR1–638 cells pretreated with PD98059 still exhibited stress fiber breakdown upon hGH stimulation similar to that of the vehicle-treated cells (Fig. 9C). These results suggest that the stress fiber breakdown stimulated by hGH is mediated specifically through the p38 MAP kinase pathway.

STAT5-mediated Transcriptional Activation Is Independent of p44/42 MAP Kinase and p38 MAP Kinase—One major mechanism by which hGH affects cellular function is by use of the JAK-STAT pathway, especially JAK2 and STAT5 (1, 2). We have demonstrated here that the p38 MAP kinase pathway is JAK2-dependent, and therefore, we wished to determine if p38 MAP kinase was therefore also required for STAT5 activation. CHO-GHR1–638 cells were transfected with the SPl-GLE1-LUC plasmid and pCMVβ vector and were pretreated with 30 μM PD98059 or SB203580 for 45 min before subsequent stimulation with 50 nM hGH for 5 min and fixed in 4% paraformaldehyde as described under “Experimental Procedures.” filamentous actin was visualized with phalloidin-TRITC and analyzed by confocal laser scanning microscopy. Similar results were obtained in at least three separate experiments.
Roles of p44/42 MAP Kinase and p38 MAP Kinase Pathways in hGH-induced Mitogenesis—To characterize the roles of p38 MAP kinase and p44/42 MAP kinase pathways in hGH-induced cell mitogenesis, we examined the effect of specific chemical inhibitors of the p44/42 MAP kinase and p38 MAP kinase pathways on hGH-induced cell proliferation in CHO-GHR1–638 cells. Cell proliferation was estimated by the \([^{3}H]\)thymidine incorporation assay. The effects of these inhibitors on DNA synthesis were first examined over a range of inhibitor concentrations with or without the presence of 50 nM hGH as indicated. Thus we observed that both PD98059 and SB203580 inhibit hGH-stimulated \([^{3}H]\)thymidine incorporation in a dose-dependent manner (Fig. 10, A and B). We next examined \([^{3}H]\)thymidine incorporation over a range of hGH concentrations when the concentration of inhibitors was held constant (Fig. 10C). Inhibition of MEK1 by PD98059 attenuated hGH-induced DNA synthesis by 40–45% in CHO-GHR1–638 cells. A similar pattern of attenuation of hGH-induced CHO-GHR1–638 cell proliferation was observed upon inhibition of p38 MAP kinase by SB203580. A combination of both inhibitors resulted in an additive effect on the inhibition of cell proliferation, reducing hGH-induced DNA synthesis by 60–70% compared with cells treated with Me2SO alone.

DISCUSSION

In the present study we have demonstrated that hGH induces the rapid phosphorylation and activation of p38 MAP kinase. We (13, 54) and others (55, 56) have previously demonstrated that other members of the MAP kinase family, namely p44/42 MAP kinase and c-Jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK), are also activated by GH. In support of our demonstration of GH-dependent activation of p38 MAP kinase, the groups of Schwartz and Carter-Su (73) have recently reported a minimal (0.6-fold) increase in p38 MAP kinase activity after GH stimulation of 3T3-F442A cells. The role of activation of the MAP kinases in the cellular effects of GH requires further delineation. It has been demonstrated that GH activation of p44/42 MAP kinase is required for the hyperproliferation of mammary carcinoma cells induced by autocrine production of GH (82). We have demonstrated here that p38 MAP kinase is required for hGH stimulation of ATF-2- and CHOP-mediated transcriptional activation, for hGH-stimulated reorganization of the actin cytoskeleton, and also for hGH-stimulated mitogenesis. Thus it is apparent that GH activation of p38 MAP kinase is pivotal in mediation of the pleiotropic cellular effects of GH.

It is apparent that GH stimulation of p38 MAP kinase activity is JAK2-dependent. JAK2 has been proposed to be the initial event in GH signal transduction (1, 4, 83), and no GH-dependent tyrosine phosphorylation of proteins is observed in cells transfected with cDNAs encoding GH receptor mutations lacking the ability to activate JAK2 (58). JAK2 has also been demonstrated to be required for GH-dependent activation of p44/42 MAP kinase and a variety of other signaling molecules including p125FAK (12), IRS-1, and IRS-2 (6). In the case of p44/42 MAP kinase, JAK2 is required for the phosphorylation of SHC and Grb-2 (8) and therefore subsequent coupling of Ras-Raf upstream of MEK1 (5, 58, 83). Interestingly though, JAK2 is apparently not required for GH stimulation of calcium

Fig. 10. Role of p44/42 MAP kinase and p38 MAP kinase pathways in hGH-induced mitogenesis. Cell proliferation was estimated by the \([^{3}H]\)thymidine incorporation assay. The effects of the inhibitors on DNA synthesis were first examined over a range of concentrations of the inhibitors in the presence of 50 nM hGH in CHO-GHR1–638 cells. \([^{3}H]\)Thymidine incorporations were also examined in CHO-GHR1–638 cells untreated or pretreated with Me2SO (○), 30 µM PD98059 (○), 10 µM SB203580 (△), or a combination of 30 µM PD98059, 10 µM SB203580 (□) for 45 min and stimulated with the range of indicated hGH concentrations (○). Experiments were performed in triplicate, and the results from three independent experiments are shown; S.E. values were below 10%.
ion influx through L-type calcium channels (84). In any case this is the first demonstration that p38 MAP kinase is activated in a JAK-dependent manner despite the fact that p38 MAP kinase has been reported to be activated by other members of the cytokine receptor superfamily which also utilize JAK2 such as erythropoietin (85) and granulocyte colony-stimulating factor (19). It is most probable that JAK2 is upstream of a previously described pathway for the activation of p38 MAP kinase (3). We (12) and others (86) have placed the focal adhesion protein-tyrosine kinase family (including FAK and Pyk2) as downstream components of JAK activation (FAK downstream of JAK2 and Pyk2 downstream of JAK3). It has recently been reported that Pyk2 is critical for the JAK-mediated p44/42 MAP kinase (87) and p38 MAP kinase (39) activation, and therefore a JAK2-FAK coupling may be one mechanism for GH activation of the p38 MAP kinase pathway. In this regard it is interesting that GH utilizes JAK2-dependent phosphorylation of the EGF receptor to activate p44/42 MAP kinase (89).

ATF-2 is one member of the ATF/CREB family of transcription factors and binds to the cAMP response element as a homodimer or heterodimer with c-Jun (90). Interestingly, GH has previously been demonstrated to increase the cellular level of c-Jun resulting in increased binding to AP-1 sites (91). The amino-terminal region of ATF-2 contains the transcriptional activation domain, which is phosphorylated on Thr-69, Thr-71, and Ser-90 by stress-activated kinases (including p38 MAP kinase) (3, 23, 42) leading to increased ATF-2 trans-activation (42, 90). We have demonstrated here that hGH stimulation of cells results in phosphorylation of ATF-2 and subsequent ATF-2-mediated transcriptional activation. As could be expected from the JAK2-dependent activation of p38 MAP kinase, we also demonstrated that ATF-dependent. ATF-2 trans-activation stimulated by GH may also provide a mechanism for the increase in c-Jun transcription noted after cellular exposure to GH since ATF-2 is found in a multiprotein complex with p300 binding to the Jun2 element of the c-jun promoter (92). ATF-2 trans-activation may also provide one mechanism for the p38 MAP kinase-dependent portion of GH-stimulated cell proliferation since ATF-2 has been demonstrated to cooperate with v-Jun to promote cell proliferation (93).

We have also demonstrated here that hGH stimulation of cells results in the transcriptional activation of CHOP. CHOP, also known as GADD153 (growth arrest and DNA damage), is a mammalian gene that encodes for a small nuclear protein related to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors (44). We have demonstrated here that the hGH-stimulated transcriptional activation of CHOP is p38 MAP kinase-dependent. This is concordant with the demonstration that CHOP undergoes stress-induced phosphorylation on two adjacent serine residues (78 and 81) by p38 MAP kinase in vivo with a resultant enhancement of CHOP trans-activation (44). CHOP was initially proposed to be involved in cell cycle arrest and apoptosis (94–96). It is therefore interesting that we observed hGH-stimulated proliferation, both here in CHO-GHR1–638 cells and in hGH-producing mammary carcinoma cells (82), to be p38 MAP kinase-dependent. Autocrine production of hGH in mammary carcinoma cells results in the transcriptional up-regulation of CHOP and increased CHOP-mediated transcription. Overexpression of CHOP by stable transfection of CHOP cDNA in mammary carcinoma cells results in enhanced cell proliferation in response to hGH, suggesting that CHOP is also a positive regulator of cell number.

Recent evidence derived from in vivo models of CHOP deficiency indicates that CHOP may possess a dual function by regulation of both apoptosis and cellular regeneration (97). It has been observed that CHOP protein influences gene expression both as a dominant negative regulator of C/EBP binding to one class of DNA targets and positively by directing CHOP-C/EBP heterodimers to other sequences (98), and this observation may constitute the mechanism of its apparent dichotomous function. hGH may therefore utilize CHOP as a positive regulator of gene transcription with resultant mitogenesis in the cell systems described. It is also interesting that GH itself has also been reported to be both mitogenic and growth-inhibitory depending on the cellular context (1, 2).

We have demonstrated here that both p38 MAP kinase and p44/42 MAP kinase cooperate during hGH-stimulated mitogenesis in the CHO-GHR1–638 cell line. Such cooperation between p38 MAP kinase and P44/42 MAP kinase has also been observed for granulocyte colony-stimulating factor stimulated proliferation of hemopoietic cells (19). Granulocyte colony-stimulating factor is another member of the cytokine receptor superfamily (1–3). Interestingly, however, we did not observe such cooperativity between p38 MAP kinase and p44/42 MAP kinase in mammalian carcinoma cells with autocrine hGH production, as the autocrine hGH-stimulated cell proliferation was completely inhibited with either the inhibitor for MEK1 (PD98059) or the inhibitor for p38 MAP kinase (SB203580) (82). The target molecules activated by either p38 MAP kinase or p44/42 MAP kinase responsible for the cooperative effect on proliferation in the CHO-GHR1–638 cell line are not defined. Presumably, however, they must represent a class of molecules, which are exclusively activated by GH stimulation of either p38 MAP kinase or p44/42 MAP kinase. Such an example may be provided in this paper whereby the GH-stimulated activation of ATF-2 and CHOP were p38 MAP kinase-dependent, whereas GH-stimulated activation of Elk-1 was p44/42 MAP kinase-dependent (73). GH has been reported to utilize Elk-1 to mediate GH-induced transcription of egr-1 (99) which may provide a mechanism for the p44/42 MAP kinase-dependent component of GH-stimulated proliferation. GH is a comparatively weak mitogen, and many effects of GH are exerted on differentiated cell function. GH may therefore also utilize p38 MAP kinase for regulation of differentiation or differentiated cell function. For example, p38 MAP kinase has been reported to be required for erythroid differentiation (100), chondrogenesis (101), and myotube formation (102).

We have demonstrated here that inhibition of p38 MAP kinase prevents hGH-stimulated stress fiber breakdown in the CHO-GHR1–638 cell line, whereas inhibition of MEK1 was without effect. Previous reports (80, 103, 104) have demonstrated that modulation of actin dynamics by p38 MAP kinase requires the phosphorylation of HSP27 downstream of MAPKAP-2 and MAPKAP-3 (80, 103–105). MAPKAP-2 has previously been reported to be activated by GH (73). It is therefore likely that GH also modulates the phosphorylation status of HSP27, although this has not yet been demonstrated. GH-stimulated re-organization of the actin cytoskeleton has been demonstrated to require phosphatidylinositol 3-kinase 3-kinase activity (79), suggesting that phosphatidylinositol 3-kinase may also be upstream of the GH-dependent increase in p38 MAP kinase activity. A previous study has reported that p38 MAP kinase activated by the chemotactic peptide N-formyl-Met-Leu-Pho requires phosphatidylinositol 3-kinase activity (106). Such is the case for hGH stimulation of both p44/42 MAP kinase (73, 110) and JNK/SAPK.²

² H. C. Mertani, T. Zhu, G. Morel, K. O. Lee, and P. E. Lobie, manuscript in preparation.

³ E. L. Goh, T. Zhu, and P. E. Lobie, manuscript in preparation.
In summary we have demonstrated here that hGH transiently phosphorylates and activates p38 MAP kinase in CHO cell lines stably transfected with rat GH receptor cDNA and that the activation of the p38 MAP kinase pathway by hGH is JAK2-dependent. Furthermore, both ATF2 and CHOP are transcriptionally activated by hGH in a JAK2-dependent manner, and p38 MAP kinase is required for both cytokoskeletal re-organization and cell proliferation stimulated by hGH. Since p38 MAP kinase has also been reported to phosphorylate and activate several other protein kinases, including MNK1, MNK2, MAPKAPK2, MAPKAPK3, MSK1, and PRAK (22, 52, 108–112), it is likely to be central to the pleiotropic cellular effects of hGH.

Acknowledgments—We thank Drs. Nils Billestrup, Shenggou Lin, and Jianhua Han for contribution to this work.

REFERENCES

1. Carter-Su, C., and Smill, L. S. (1988) Recent Prog. Horm. Res. 53, 61–82
2. Lobie, P. E. (1999) in Growth Hormone (Bentgson, B. A., ed.), pp. 17–35, Kluwer Academic Publishers, Norwell, MA
3. Ebi, M., and Hiraoka, T. (1998) Int. Rev. Immunol. 17, 75–102
4. Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Bile, J. N., and Carter-Su, C. (1999) Cell 74, 237–244
5. Winston, L. A., and Hunter, T. (1995) J. Biol. Chem. 270, 30837–30840
6. Yoshizaki, T., Kajimura, Y., Ueki, K., Tsuji, Y., Stark, G. R., Kerr, I. M., Tsushima, T., Akunama, Y., Komuro, I., Tobe, K., Yazaki, Y., and Kadokawi, T. (1998) J. Biol. Chem. 273, 15179–15176
7. Ridderstad, M., Degenmar, E., and Tornqvist, H. (1995) J. Biol. Chem. 270, 3471–3474
8. VanderKuur, J., Allevato, G., Billestrup, N., Norstedt, G., and Carter-Su, C. (1995) J. Biol. Chem. 270, 7587–7592
9. Rui, L., Mathews, L. S., Hotta, K., Gustafson, T. A., and Carter-Su, C. (1998) J. Biol. Chem. 273, 6633–6644
10. Ram, P. A., and Waxman, D. J. (1997) J. Biol. Chem. 272, 17694–17702
11. Kim, S. O., Irwin, P., Katz, S., and Pelech, S. L. (1997) J. Biol. Chem. 272, 4028–4038
12. Zetter, A., Gredinger, E., and Bengal, E. (1999) J. Biol. Chem. 274, 5193–5200
13. Yang, S. H., Galanis, A., and Sharrackks, A. D. (1999) Mol. Cell. Biol. 19, 10583–10587
14. New, L., Jiang, Y., Zhao, M., Liu, K., Zhu, W., Flood, L. J., Kato, Y., Parry, G. C., and Han, J. (1998) J. Biol. Chem. 273, 3372–3384
15. McLaughlin, M. M., Kumar, S., McDonnell, P. C., Van Horn, S. L., Lee, J. C., Livin, P. G., and Young, P. R. (1999) J. Biol. Chem. 274, 8485–8492
16. Muller, C., Hannon, A., Enberg, B., Loebe, P. E., and Norstedt, G. (1992) J. Biol. Chem. 267, 23403–23408
17. Harding, P. A., Wang, X. Z., and Kopchick, J. J. (1995) Receptor S 81–92
18. Lobie, P. E., Roncin, B., Silvennoinen, O., Raldhousen, L. A., Norstedt, G., and Schwartz, J. M. (1996) Endocrinology 139, 4654–4667
19. Wood, T. J., Sliva, D., Lobe, P. E., Goulieux, F., Mui, A. L., Groen, B. N., Norstedt, G., and Haldosén, O. L. (1997) Mol. Cell. Endocrinol. 139, 69–81
20. VanderKuur, J., Awan, Z., Zhang, L., Campbell, G. S., Allevato, G., Billestrup, N., Norstedt, G., and Carter-Su, C. (1994) J. Biol. Chem. 269, 21709–21717
21. Tannenbaum, G. S., and Martin, J. B. (1999) Endocrinology 195, 582–570
22. Eisman, K. D., Schwartz, J., and Ketels, J. L. (1998) Mol. Endocrinol. 12, 1541–1552
23. Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Lubi, P. E. (1999) Mol. Cell. Biol. 19, 4028–4038
25. Lobie, P. E. (1999) Endocrinology 139, 4364–4372
26. Brunet, A., and Pouyssegur, J. (1999) Science 282, 1652–1655
27. Metalnikov, N., Kramm, G., Yumaguzin, A., Lapidus, E., Lapidus, Z., Leeder, J. S., Freedman, M., Cohen, A., Gaffes, A., Letzgus, A., and Roisman, C. M. (1999) Nature 379, 645–648
28. Harris, K. W., Hu, J. X., Schultz, S., Arcosotti, M. O., Forget, B. G., and Clare, N. (1998) Blood 92, 1211–1217
29. Eisman, K. D., Schwartz, J., and Ketels, J. L. (1998) Mol. Endocrinol. 12, 1541–1552
30. Abe, J., and Berk, B. C. (1999) J. Biol. Chem. 274, 21003–21010
31. Kortenjann, M., Thomae, O. and Shaw, P. E. (1994) Mol. Cell. Biol. 14, 4815–4824
32. Hill, C. S., and Treisman, R. (1995) Cell 80, 199–211
33. Deleted in proof
34. Hodge, C., Liao, J., Stufeg, M., Guan, K., Carter-Su, C., and Schwartz, J. (1998) J. Biol. Chem. 273, 5137–5136
35. Pang, L., Sawada, T., Decker, S. J., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 15385–15388
36. Alesi, D., Cuenca, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 24739–24744
37. Wang, X. Z., and Ron, D. (1996) Science 272, 1347–1349
38. Albert, D., Schwenger, P., Han, J., and Vikeek, J. (1999) J. Biol. Chem. 274, 22176–22183
39. Yang, S. H., Whitmarsh, A. J., Davis, J. R., and Sharracks, A. D. (1998) EMBO J. 17, 1474–1479
40. Gol, E. L., Fischer, T. T., Wood, T. J., Norstedt, G., Graichen, R., and Lobe, P. E. (1997) Endocrinology 138, 3207–3215
41. Larsen, J. K., Yamabay, I. A., Weber, L. A., and Gerthoffer, W. T. (1997) J. Biol. Chem. 272, 1387–1396
42. Schafer, C., Ross, S. E., Grabeaud, M. G., Groblewski, G. E., Ernst, S. A., and Williams, J. A. (1998) J. Biol. Chem. 273, 24173–24180
43. Kauls, K., Mertani, H., Tornell, J., Morel, G., Lee, K. O., and Lobie, P. E. (1999) Exp. Cell Res. 254, 230–239
44. VanderKuur, J. A., Butch, E. R., Waters, S. B., Pessin, J. E., Guan, K. L., and Vanderkooi, J. A. (1998) Endocrinology 139, 2077–2088
45. Nagata, Y., Moriguchi, T., Nishida, E., and Tokodoko, K. (1997) Blood 90, 2139–2149
46. Miyazaki, T., Takaoka, A., Nogueria, L., Ikida, K., Fujii, H., Tsujino, S., Mitani, Y., Maeda, M., Schlessinger, J., and Taniguchi, T. (1998) Genes Dev. 12, 770–775
47. Takaoka, A., Tanaka, N., Mitani, Y., Miyazaki, T., Fujii, H., Sato, M., Koevick, A., Takaoka, A., Tanaka, N., Mitani, Y., Miyazaki, T., Fujii, H., Sato, M., Koevick, A.
