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# Mediation of Growth Hormone-dependent Transcriptional Activation by Mammary Gland Factor/Stat 5\*

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Previous observations have shown that binding of growth hormone to its receptor leads to activation of transcription factors via a mechanism involving phosphorylation on tyrosine residues. In order to establish whether the prolactin-activated transcription factor Stat 5 (mammary gland factor) is also activated by growth hormone, nuclear extracts were prepared from COS-7 cells transiently expressing transfected Stat 5 and growth hormone receptor cDNA. Gel electrophoresis mobility shift analyses revealed the growth hormone-dependent presence of specific DNA-binding proteins in these extracts. The complexes formed could be supershifted by polyclonal anti-Stat 5 antiserum. In other experiments nuclear extracts from growth hormone-treated Chinese hamster ovary cells stably expressing transfected growth hormone receptor cDNA and liver from growth hormone-treated hypophysectomized rats were used for gel electrophoresis mobility shift analyses. These also revealed the presence of specific DNA-binding proteins sharing antigenic determinants with Stat 5. Stat 5 cDNA was shown to be capable of complementing the growth hormone-dependent activation of transcription of a reporter gene in the otherwise unresponsive COS-7 cell line. This complementation was dependent on the presence of Stat 5 tyrosine 694, suggesting a role for phosphorylation of this residue in growth hormone-dependent activation of DNA-binding and transcription.

Pituitary growth hormone is the principal hormone regulating growth in mammals (1). However, in addition to its unique somatogenic effects, growth hormone also shares some of the lactogenic properties of its close relative prolactin: stimulating both mammary gland development (2, 3) and lactation (4). Both growth hormone and prolactin regulate cellular processes as a result of their binding to specific membrane-bound receptors (5–8). These receptors have been cloned from a variety of species and assigned to a family of peptide hormone and growth factor receptors, the cytokine receptor superfamily. This family, which includes receptors for prolactin, interleukins-2

through -7, erythropoietin, and granulocyte- and granulocyte-macrophage colony-stimulating factors, is characterized by structural features such as a single membrane spanning domain, an arrangement of four conserved extracellular cysteine residues, the absence of intrinsic protein kinase activity, and an intracellular proline-rich region (9–11). There is now increasing evidence that the cytokine receptors also share common components in their signal transduction pathways. Prominent among these are protein tyrosine kinases of the Janus kinase family (12) which have been shown to associate with a variety of cytokine receptors, including those for growth hormone and prolactin (13, 14). In addition, growth hormone, prolactin, interleukins-4 and -6, and interferon- $\gamma$ , the receptor for which bear some similarity with the other cytokine receptors (11), all appear to enhance gene transcription as a result of activation of members of the Stat family of DNA-binding proteins (15–20). These molecules are grouped together on the basis of their possession of *src* homology domains of type 2 and 3 (SH2 and SH3) and their ability to bind to short palindromic interferon- $\gamma$ -activated sequence (GAS)<sup>1</sup>-like DNA elements. Such elements are widely distributed among cytokine-activated gene promoters (21).

Growth hormone stimulation of several cell lines has been shown to activate binding of nuclear proteins to a GAS-like DNA element, the serine protease inhibitor GAS-like element 1 (SPI-GLE 1), present in the serine protease inhibitor 2.1 promoter. This in turn leads to the enhancement of expression of adjacent cDNA sequences in reporter-plasmid constructs (15). In this paper we describe the growth hormone-dependent activation of SPI-GLE 1 binding of the recently described prolactin-activated Stat, Stat 5 (mammary gland factor, Ref. 22). We also demonstrate that Stat 5 cDNA is able to facilitate the activation of a growth hormone reporter system based on the SPI-GLE 1. These results indicate that the similarities between the endocrinology of growth hormone and prolactin extend to their ability to utilize the same factor for the enhancement of gene transcription.

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<sup>1</sup> The abbreviations used are: GAS, interferon- $\gamma$ -activated sequence; SPI-GLE 1, serine protease inhibitor 2.1 GAS-like element 1; PMSF, phenylmethylsulfonyl fluoride; pGLE-LUC, luciferase reporter plasmid containing thymidine kinase promoter and SPI-GLE 1 enhancer elements; TK, thymidine kinase; pTK-LUC, luciferase reporter plasmid containing thymidine kinase promoter; BRL cells, Buffalo rat liver cells; CHO cells, Chinese hamster ovary cells; COS-7, African green monkey CV1 cells transformed with an origin-defective SV-40 mutant virus; CAT, chloramphenicol acetyltransferase; JAK: Janus (just another) kinase; DOTAP, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulfate.

## EXPERIMENTAL PROCEDURES

*Cell Culture and Animals*

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Life Technology, Inc.) and Chinese hamster ovary cells stably expressing transfected rat growth hormone receptor cDNA (designated CHO-4 cells) in Ham's F-12 Medium (Life Technology, Inc.). Both media were supplemented with 10% fetal calf serum (Life Technology, Inc.) and 50 units/ml penicillin, 50 µg/ml streptomycin (Life Technology, Inc.). Hypophysectomized Sprague-Dawley male rats were purchased from ALAB, Denmark and maintained on standard chow.

*Preparation of Antisera*

Polyclonal Stat 5 "antiserum" was raised, using the chicken egg yolk system, against a Stat 5-glutathione *S*-transferase fusion protein containing Stat 5 amino acid residues 6–132. This antiserum was found to be specific for Stat 5 in so far as it did not recognize either Stat 1 or Stat 3 in gel electrophoresis mobility shift assays.<sup>2</sup>

*Preparation of Cell Extracts*

**CHO-4 Cells**—CHO-4 cells were cultured as described and then prior to addition of 100 nM recombinant human growth hormone (Pharmacia) were starved of fetal calf serum for 12–20 h. After treatment, the cultures ( $1-2 \times 10^7$ /extract) were chilled on ice, rinsed with ice-cold phosphate-buffered saline, and nuclear extracts prepared. The cells were scraped into 10 ml of resuspension buffer containing 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 6 mM MgCl<sub>2</sub>, 1 mM DTT, 0.4 mM PMSF, and 0.1 mM Na<sub>3</sub>VO<sub>4</sub> and then homogenized in a Dounce type B homogenizer. The nuclear pellet, after centrifugation, was resuspended in 3 volumes of lysis buffer containing 20% glycerol, 20 mM Hepes, pH 7.9, 420 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.2 mM PMSF, 1 mM DTT, and 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, and incubated on ice for 30 min. Further cell debris was removed by centrifugation and the supernatant nuclear extract collected for gel electrophoresis mobility shift assays.

**COS-7 Cells**—COS-7 cells were cultured as described and then co-transfected with expression plasmids for the growth hormone receptor and Stat 5. Transfections were carried out with DOTAP (Boehringer Mannheim) using  $2 \times 10^7$  cells for each extract together with 10 µg of each CsCl density gradient purified expression plasmid, following the manufacturer's instructions. After transfection cells were incubated in serum free media for 48 h and then exposed to 100 nM human growth hormone for 15 min. Whole cell extracts were then prepared. Cells were scraped into 1.5 ml of hypotonic lysis buffer containing 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 6 mM MgCl<sub>2</sub>, 1 mM DTT, 0.4 mM PMSF, and 0.1 mM Na<sub>3</sub>VO<sub>4</sub>. The extract was then centrifuged, the supernatant discarded, and the cell pellet incubated for 45 min in 3 volumes of a buffer containing 20% glycerol, 20 mM Hepes, pH 7.9, 420 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.2 mM PMSF, 1 mM DTT, 0.1 mM Na<sub>3</sub>VO<sub>4</sub>. Further cell debris was removed by centrifugation and the supernatant collected for gel electrophoresis mobility shift assays.

**Rat Liver**—Six hours or 2 h prior to sacrifice male, hypophysectomized, Sprague-Dawley rats were given a single subcutaneous injection of 25 µg of bovine growth hormone. Following sacrifice of the rats by decapitation, livers were removed, minced, and frozen in liquid nitrogen. Each nuclear extract was prepared from 2 g of liver. The liver was disrupted using a Polytron in 2 ml of lysis buffer containing 10 mM Hepes, pH 7.9, 25 mM NaCl, 0.15 mM spermine, 10% glycerol, and 1 mM EDTA. A further 7 ml of lysis buffer was added and homogenization continued with 10 strokes in a Dounce homogenizer. Nuclei were then isolated by centrifugation at  $100,000 \times g$  for 30 min, resuspended in 3 volumes of extraction buffer containing 10 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM DTT, 0.1 mM PMSF, and incubated for 1 h at 4 °C. The extract was finally centrifuged at  $10,000 \times g$  and the supernatant collected and frozen at -70 °C.

*Gel Electrophoresis Mobility Shift Assay*

Nuclear extracts were incubated with <sup>32</sup>P-labeled double-stranded SPI-GLE 1: (TGTTCTGAGAAATA) oligonucleotides (core sequence shown underlined), in 15 µl of a buffer containing 4% Ficoll, 12 mM Hepes, pH 7.9, 4 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 1 mM DTT, and 5 µg of poly(dI-dC).

*Assembly of Reporter Constructs*

Double-stranded oligonucleotides containing three tandem SPI-GLE 1 sequences: CTAGTGTCTGAGAAATGAACGGTTCTGAGAAAGTA-

CAGGTCTGAGAAAT (SPI-GLE 1 elements shown underlined) were ligated together with a minimal thymidine kinase (TK) promoter into the pGL2 plasmid (Promega), which contains the firefly luciferase cDNA, to make pGLE-LUC. The TK promoter alone was ligated into pGL2 to give the control plasmid pTK-LUC.

*Cell Transfection with Reporter Plasmid and Luciferase Assay*

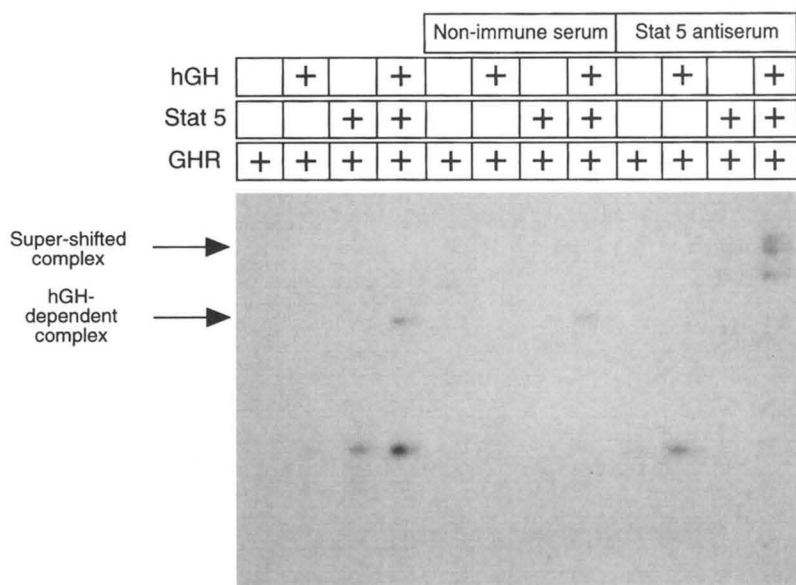
COS-7 cells were cultured in 30-mm dishes to ~75% confluence and then washed twice with phosphate-buffered saline. Transfections were carried out in serum-free Dulbecco's modified Eagle's medium with DOTAP (Boehringer Mannheim) according to the manufacturer's instructions using, in various combinations, the reporter plasmids pGLE-LUC or pTK-LUC and cDNA expression plasmids for: (i) wild type Stat 5, (ii) Tyrosine 694 → phenylalanine Stat 5 mutant, (iii) rat growth hormone receptors, (iv) mouse prolactin receptors (long form), and (v) chloramphenicol acetyltransferase (CAT). All plasmids had been purified by CsCl density gradient centrifugation. Cells were incubated with DOTAP/DNA for 12 h and then the media was changed to serum-free Dulbecco's modified Eagle's medium containing 100 nM human growth hormone. Human, unlike bovine, growth hormone binds to both the rodent prolactin and growth hormone receptors with high affinity (23) and was thus considered a suitable substitute for mouse prolactin. Binding assays were performed as described (24), and untransfected COS-7 cells were found to express less than 1% of the human growth hormone binding activity of COS-7 cells which had been transiently transfected with rat growth hormone receptor cDNA. After a further 12 h, cells were washed with phosphate-buffered saline and then scraped into lysis buffer containing 25 mM Tris phosphate, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid, 10% glycerol, and 1% Triton X-100. CAT assays were performed by incubating 30 µl of extract with 3 µl of [<sup>14</sup>C]chloramphenicol (Amersham Corp., 50–62 mCi/mmol, 25 mCi/ml) and 5 µl of 5 mg/ml butyryl coenzyme A (Boehringer Mannheim) for 3 h at 37 °C. Butyrylated chloramphenicol was then extracted with xylene and detected using a Wallac scintillation counter. Luciferase assays were performed using a BioOrbit luminometer with a pump dispenser. 30 µl of extract was added to 300 µl of buffer containing 0.12 mg/ml D-luciferin, 10 mM DTT, 1 mg/ml bovine serum albumin, 10 mM magnesium acetate, 0.1 M Tris acetate, pH 7.75, and 2 mM EDTA. Luciferase measurements were started by injecting 30 µl of 5 mM ATP, 0.5 mM sodium pyrophosphate and continued for 30 s. The integrated signal was taken as representative of luciferase activity.

## RESULTS

We have shown previously that growth hormone treatment of Buffalo rat liver and Chinese hamster ovary cells that have been stably transfected with rat growth hormone receptor cDNA (designated BRL-4 and CHO-4 cells, respectively) causes the rapid activation of nuclear protein binding activity to an 11-base pair DNA element, the serine protease inhibitor  $\gamma$ -activated sequence-like element 1 (SPI-GLE 1, Ref. 15). SPI-GLE 1 is partly responsible for the growth hormone-responsiveness of a region of the serine protease 2.1 promoter and shares homology with DNA elements found in promoters activated by interferon- $\gamma$ , interleukin-6, and prolactin (21). Following publication of results revealing that the prolactin-activated mammary gland factor DNA-binding protein is a member of the Stat family (22), and bearing in mind the similarities between growth hormone and prolactin's mechanisms of action, we were interested to see whether this molecule, Stat 5, is activated by growth hormone with respect to SPI-GLE 1 binding. COS-7 cells were co-transfected with growth hormone receptor and Stat 5 cDNA or growth hormone receptor cDNA alone, treated for 10 min with human growth hormone, a regime which has been shown previously to elicit maximal SPI-GLE 1 binding activity, and then harvested for preparation of nuclear extracts. Gel electrophoresis mobility shift assays revealed that only cells which had been transfected with both Stat 5 and growth hormone receptor cDNA and then growth hormone-treated contained specific SPI-GLE 1-binding proteins (Fig. 1). A nonspecific band of greater mobility than the specific band was sometimes observed. The presence of this band did not appear to depend upon any particular treatment regime. Co-

<sup>2</sup> H. Wakao and F. Gouilleux, unpublished observation.

**FIG. 1. Growth hormone activates binding of Stat 5 to SPI-GLE 1.** COS-7 cells were transiently transfected with growth hormone receptor cDNA (*GHR*) either in the presence or absence of Stat 5 cDNA. Following transfection cells were treated with 100 nM human growth hormone (*hGH*) and whole cell extracts prepared for gel electrophoresis mobility shift assays. Extracts were co-incubated with non-immune serum or polyclonal Stat 5 antiserum as indicated.

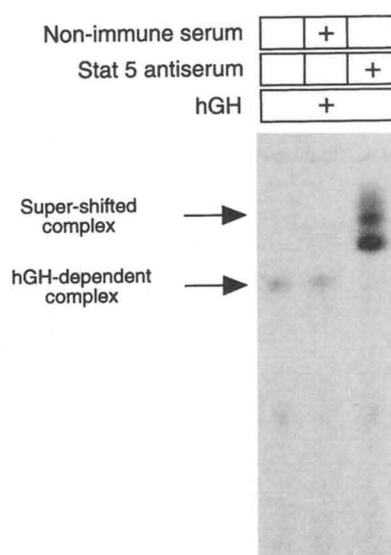


incubation of nuclear extracts during gel shift assays with polyclonal antisera raised against a Stat 5-glutathione *S*-transferase fusion protein confirmed that the growth hormone-dependent complex formed contained Stat 5.

Having shown that growth hormone is capable of activating Stat 5 in transfected COS-7 cells, it was of interest to determine whether the endogenous growth hormone-activated DNA binding protein seen in other cells shares antigenic determinants with Stat 5. We have described previously (15) the specific growth hormone-dependent activation of SPI-GLE 1-binding in growth hormone receptor transfected Chinese hamster ovary cells (CHO-4 cells). We therefore subjected nuclear extracts prepared from human growth hormone-treated CHO-4 cells to gel electrophoresis mobility shift assays in the presence or absence of polyclonal Stat 5 antiserum using the SPI-GLE 1 probe. A distinct supershift in the mobility of the observed DNA binding activity was seen in the presence of the polyclonal anti-Stat 5 antisera but not preimmune sera (Fig. 2), indicating that growth hormone does indeed activate the DNA binding of Stat 5 or a similar molecule in CHO-4 cells. No SPI-GLE 1 binding is seen in extracts from untreated CHO-4 cells (15).

Northern blot analysis has revealed that the expression of Stat 5 is not restricted to mammary gland but rather ubiquitous (22). We were therefore interested to see whether Stat 5 is present and activated by growth hormone in liver, since this is a major target for growth hormone stimulation. Nuclear extracts were prepared from livers of hypophysectomized male rats 2 or 6 h after a single injection of bovine growth hormone. Control extracts from hypophysectomized but untreated rats were also prepared. The extracts were then subjected to gel mobility shift assay with a SPI-GLE 1 probe in the presence or absence of polyclonal Stat 5 antiserum. Gel electrophoresis mobility shift assays revealed the presence of nuclear proteins capable of binding to SPI-GLE 1 only in extracts from growth hormone-treated animals (Fig. 3). These complexes contained proteins sharing antigenic determinants with Stat 5. We were, however, unable to supershift the growth hormone dependent complex in its entirety even after increasing the ratio of antiserum to nuclear proteins (data not shown). The experiment shown represents a gel mobility shift assay using approximately equivalent amounts of nuclear extract and antiserum to the analyses shown for COS-7 and CHO-4 cells.

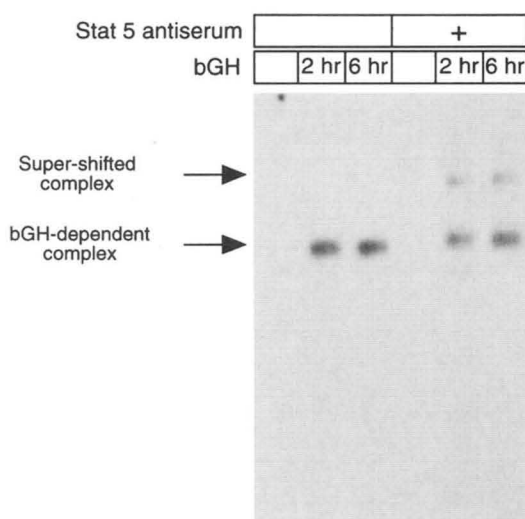
We have shown previously that SPI-GLE 1 is capable of mediating growth hormone-dependent transcriptional activa-



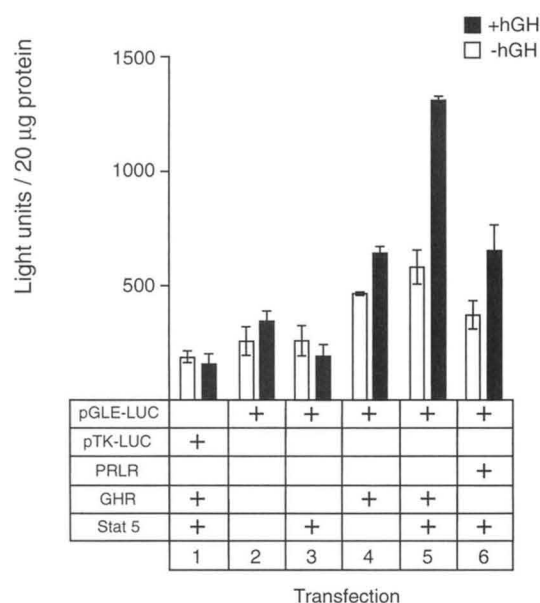
**FIG. 2. Growth hormone activates DNA-binding of a protein sharing antigenic determinants with Stat 5 in CHO-4 cells.** Nuclear extracts were prepared from CHO-4 cells which had been treated with 100 nM human growth hormone for 10 min. The extracts were subjected to gel electrophoresis mobility shift assays with a SPI-GLE 1 probe, alone and in the presence of preimmune or Stat 5 antiserum.

tion of a reporter cDNA in transfection experiments with Buffalo rat liver and Chinese hamster ovary cells stably expressing transfected growth hormone receptor cDNA (15). This is also the case for Buffalo rat liver cells transiently expressing transfected prolactin receptor cDNA.<sup>3</sup> In order to determine whether this growth hormone-dependent transcriptional activation could be mediated by Stat 5, we co-transfected COS-7 cells with expression plasmids for Stat 5 and the growth hormone or prolactin receptors together with a luciferase reporter construct either containing (pGLE-LUC) or lacking (pTK-LUC) three copies of the SPI-GLE 1. In the absence of SPI-GLE 1 elements in the reporter plasmid no growth hormone-dependent induction in luciferase expression was seen even when the cells were transfected with cDNA for growth hormone receptors and Stat 5 (Fig. 4, lane 1). Similarly low levels of activity were seen

<sup>3</sup> T. J. J. Wood *et al.*, unpublished observation.

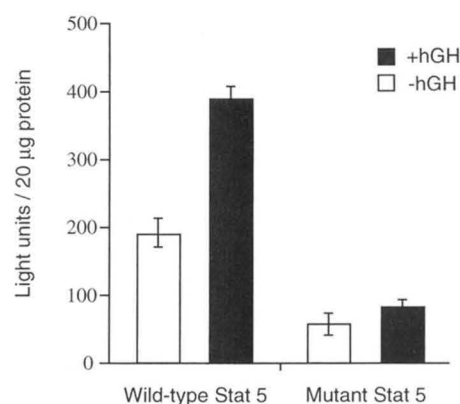


**FIG. 3. Growth hormone activates DNA-binding of a protein sharing antigenic determinants with Stat 5 in rat liver.** Nuclear extracts were prepared from the livers of control hypophysectomized rats and the livers of hypophysectomized rats 2 h or 6 h after a single injection of bovine growth hormone. The extracts were then subjected to gel electrophoresis mobility shift assays with a SPI-GLE 1 probe alone or in the presence of Stat 5 antiserum.



**FIG. 4. Stat 5 is capable of mediating growth hormone-dependent transcriptional activation.** COS-7 cells were transfected with various combinations of reporter plasmids containing or lacking SPI-GLE 1 DNA elements (*pGLE-LUC* or *pTK-LUC*, respectively) and expression plasmids for Stat 5, growth hormone receptors (*GHR*), or prolactin receptors (*PRLR*) together with a plasmid constitutively expressing chloramphenicol acetyltransferase (CAT). Each bar represents the average of three independent experiments with error bars representing standard deviations. CAT activities were measured for each sample and the variation in transfection efficiencies found to lie within the standard deviations presented.

when cells were transfected with *pGLE-LUC* either alone or in the presence of Stat 5 cDNA (lanes 2 and 3). Co-transfection of growth hormone receptor cDNA with *pGLE-LUC* resulted in higher levels of growth hormone-independent luciferase activity with a small induction of activity following hormone treatment (lane 4). Transfection of additional Stat 5 cDNA together with growth hormone receptor cDNA and *pGLE-LUC*, however, complemented the ability of COS-7 cells to respond to growth hormone and an approximately 2-fold induction of luciferase activity



**FIG. 5. Tyrosine 694 is essential for activation of Stat 5 by growth hormone.** COS-7 cells were transfected with *pGLE-LUC* and expression plasmid for growth hormone receptors (*GHR*) and mutated ( $\text{Ty}^{694} \rightarrow \text{Phe}$ ) or wild type Stat 5 together with a plasmid constitutively expressing CAT. Each bar represents the average of three independent experiments with error bars representing standard deviations. CAT activities were measured for each sample and the variation in transfection efficiencies found to lie within the standard deviations presented.

was seen following growth hormone treatment. The luciferase activity induced by human growth hormone treatment of COS-7 cells transfected with *pGLE-LUC*, and cDNA for Stat 5 and the mouse prolactin receptor is shown for comparison (lane 6). The results described here thus demonstrate that Stat 5 is capable of complementing a growth hormone response normally only weakly expressed in COS-7 cells via both the growth hormone and prolactin receptors.

Prolactin has been shown to activate DNA-binding of Stat 5 by inducing its phosphorylation on tyrosine residue 694 (25). Both growth hormone and prolactin stimulate tyrosine phosphorylation of JAK 2, a molecule which has been shown to be necessary for the activation of Stats by other cytokines and which is probably a Stat kinase. It is consequently not unreasonable to suppose that growth hormone and prolactin activate Stat 5 by the same mechanism. This hypothesis was supported by the observation that substitution of tyrosine 694 for a phenylalanine in the Stat 5 expression plasmid resulted in a loss of growth hormone dependence of luciferase expression in co-transfection experiments with COS-7 cells (Fig. 5).

#### DISCUSSION

Rapid progress has recently been made in elucidating the mechanisms of signal transduction of the cytokine receptor superfamily. It is now possible to hypothesize a working model (Fig. 6) in which, following ligand binding, membrane-bound cytokine receptors activate tyrosine kinases of the Janus kinase family. This activation leads to tyrosine phosphorylation of the JAK molecule itself, the associated receptor and members of the Stat family of DNA-binding proteins (26–30). Six members of the Stat family have been characterized to date, these being p91 and its splice variant p84 (termed Stat 1  $\alpha$  and  $\beta$ , respectively), p113 (Stat 2) (21), epidermal growth factor- and interleukin-6-activated Stat 3 (acute phase response factor, Refs. 31–33), Stat 4 (34), interleukin-4-activated interleukin-4 Stat (35), and Stat 5 (mammary gland factor, Ref. 22). Following their phosphorylation on tyrosine residues, Stat molecules dimerize, presumably as a result of phosphotyrosine-SH2 domain interactions, and a specific DNA binding complex is formed (36). A consensus Stat binding sequence, TT A/CC N NN AA, can be derived from elements found within a variety of cytokine-regulated gene promoters. How Stat-DNA binding enhances gene transcription is unclear, although Stat 5 has been shown to de-repress expression via the  $\beta$ -casein promoter by



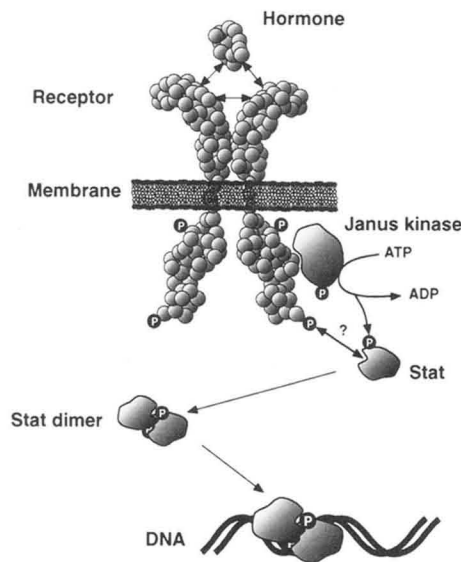


FIG. 6. A model for growth hormone-dependent activation of gene transcription via the JAK-Stat pathway.

displacing the ubiquitous transcription factor YY 1 (37, 38). The frequent observation of potential Stat binding elements in serial repeat may also be relevant to the mechanism of enhancement of transcription by the Stats (15, 39). It is not clear whether the Stats necessarily bind directly to their activating receptor, although this appears to be the case for at least interleukin-4 Stat (35) and the interleukin-4 receptor and p91/Stat 1 $\alpha$  and the epidermal growth factor receptor (40).

The gel mobility shift analyses presented here reveal that growth hormone, like prolactin, can activate the specific DNA binding of Stat 5 in transfected COS-7 cells. That this artificial system bears some similarity to the *in vivo* situation is indicated by similar experiments showing that an endogenously expressed Stat sharing antigenic determinants with Stat 5 is activated in both cultured ovary cells and liver. Further support for a role for Stat 5 in growth hormone signal transduction can be derived from the observation that transfection of Stat 5 cDNA facilitates the growth hormone-dependent activation of a reporter gene in COS-7 cells. The results obtained with COS-7 cells reveal a phenomenon seen with other cell lines of non-hepatic origin, that is a growth hormone receptor-dependent, but growth hormone-independent, activation of reporter genes (compare Fig. 4, lanes 1, 3, and 4). Such an effect is not seen in Buffalo rat liver (BRL) cells,<sup>4</sup> thus explaining why a significantly larger induction of activity is seen following growth hormone treatment of BRL-4 cells than following identical treatment of COS-7 cells (15).

Growth hormone and prolactin have been shown previously to share similarities both in terms of their physiological effects on mammary gland development and their common use of JAK 2 in signal transduction. We have now demonstrated that the similarities in the endocrinology of these two hormones extends to their ability to activate the transcription factor Stat 5 via, in both cases, a mechanism requiring the presence of Stat tyrosine 694. How these similarities can be reconciled with the unique effects of growth hormone and prolactin is as yet unclear. It appears paradoxical that prolactin induces luciferase expression via the SPI-GLE 1 in prolactin receptor cDNA-transfected cell lines but does not induce SPI 2.1 mRNA expression in hepatocytes (41).

The activation of a single Stat member by several cytokines is

not unique to Stat 5. Several different molecules, including interferon- $\gamma$ , epidermal growth factor, interleukin-6, and indeed both growth hormone and prolactin, activate a Stat recognized by polyclonal antisera to p91 (17, 20, 32, 42). It may be the case that specificity depends upon the design of the sequence flanking the Stat response element, the presence of other transcription factors in the DNA-bound complex, or the cellular expression or tyrosine phosphorylation patterns of receptors, JAKs and Stats. That Stat 5 tyrosine 694 appears to be the target for both prolactin and, as shown here, growth hormone-induced phosphorylation is not consistent with the two hormones retaining their specificity via differential Stat phosphorylation patterns. However, the observation that the growth hormone-activated Stats derived from NIH 3T3 (17) cells, but not CHO-4, BRL-4, and IM-9 (15, 43) cells, are recognized by antibodies capable of recognizing interferon- $\gamma$ -activated p91 suggest that differential expression could be important.

Several cytokines, including growth hormone, prolactin, and interleukin-6, are capable of simultaneously activating more than one different Stat family member (16, 32, 42). The formation of cell type-specific patterns of homo- and heteromeric Stat multimers is therefore an additional potential source of specificity. There are several lines of published evidence to support this hypothesis. Glycerol gradient analyses indicate that tyrosine phosphorylation induces homo- and heterodimerization of both p91 and p84 (36). In addition, interferon- $\alpha$  stimulates the formation of a DNA binding complex consisting of a heteromer containing p84/p91 (Stat1), p113 (Stat 2), and a 48-kDa DNA-binding protein (44). We observed that it was not possible to fully supershift the complexes formed between SPI-GLE 1 and nuclear proteins extracted from growth hormone-treated hypophysectomized rat liver using the same conditions capable of inducing a complete shift with CHO-4 cells. This suggests that in addition to Stat 5 other, as yet uncharacterized, transcription factors are activated by growth hormone in rat liver. We are currently investigating this possibility.

#### REFERENCES

- Isaksson, O. G. P., Eden, S. & Jansson, J.-O. (1985) *Annu. Rev. Physiol.* **47**, 483–499
- Feldman, M., Ruan, W., Cunningham, B. C., Wells, J. A. & Kleinberg, D. L. (1993) *Endocrinology* **133**, 1602–1608
- Plaut, K., Ikeda, M. & Vonder Haar, B. K. (1993) *Endocrinology* **133**, 1843–1848
- Barber, M. C., Clegg, R. A., Vernon, R. G. & Flint, D. J. (1992) *J. Endocrinol.* **135**, 195–201
- Matthews, L. S., Enberg, B. & Norstedt, G. (1989) *J. Biol. Chem.* **264**, 9905–9910
- Leung, D. W., Spenser, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Henzel, W. J., Barnard, R., Waters, M. J. & Wood, W. I. (1987) *Nature* **330**, 537–543
- Boutin, J.-M., Jolicoeur, C., Okamura, H., Gagnon, J., Edery, M., Shirota, M., Banville, D., Dusanter-Fourt, I., Djiane, J. & Kelly, P. A. (1988) *Cell* **53**, 69–77
- Shirota, M., Banville, D., Ali, S., Jolicoeur, C., Boutin, J.-M., Edery, M., Djiane, J. & Kelly, P. A. (1990) *Mol. Endocrinol.* **4**, 1136–1143
- Cosman, D., Lyman, S. D., Idzerda, R. L., Beckman, M. P., Park, L. S., Goodwin, R. G. & March, C. J. (1990) *Trends Biochem. Sci.* **15**, 265–269
- Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 6934–6938
- Thoreau, E., Petridou, B., Kelly, P. A., Djiane, J. & Mornon, J. P. (1991) *FEBS Lett.* **282**, 26–31
- Ziemiński, A., Harpur, A. G. & Wilks, A. F. (1994) *Trends Cell Biol.* **4**, 207–212
- Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N. & Carter-Su, C. (1993) *Cell* **74**, 237–244
- Dusanter-Fourt, I., Muller, O., Ziemiński, A., Mayeux, P., Drucker, B., Djiane, J., Wilks, A. G., Harpur, A. G., Fischer, S. & Gisselbrecht, S. (1994) *EMBO J.* **13**, 2583–2591
- Sliva, D., Wood, T. J. J., Schindler, C., Lobie, P. E. & Norstedt, G. (1994) *J. Biol. Chem.* **269**, 26208–26214
- Wang, X., Xu, B., Souza, S. C. & Kopchick, J. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 1391–1395
- Meyer, D. J., Campbell, G. S., Cochran, B. H., Argetsinger, L. S., Lerner, A. C., Finbloom, D. S., Carter-Su, C. & Schwartz, J. (1994) *J. Biol. Chem.* **269**, 4701–4704
- Finbloom, D. S., Petricoin, E. F., III, Hackett, R. H., David, M., Feldman, G. M., Igarashi, K.-I., Fibach, E., Weber, M. J., Thorner, M. O., Silva, C. M. & Lerner, A. C. (1994) *Mol. Cell. Biol.* **14**, 2113–2118
- Gronowski, A. M. & Rotwein, P. (1994) *J. Biol. Chem.* **269**, 7874–7878
- Darnell, J. E., Kerr, I. M. & Stark, G. R. (1994) *Science* **264**, 1415–1421

<sup>4</sup> T. J. J. Wood *et al.*, manuscript in preparation.

21. Pellegrini, S. & Schindler, C. (1994) *Trends Biochem. Sci.* **18**, 338–342
22. Wakao, H., Gouilleux, F. & Groner, B. (1994) *EMBO J.* **13**, 2182–2191
23. Shiu, R. P. C. (1979) *Cancer Res* **39**, 4381–4386
24. Emtner, M., Mathews, L. S. & Norstedt, G. (1990) *Mol. Endocrinol.* **4**, 2014–2020
25. Gouilleux, F., Wakao, H., Mundt, M. & Groner, B. *EMBO J.* **13**, 4361–4369
26. Larner, A. C., David, M., Feldman, G. M., Igarashi, K.-I., Hacket, R. H., Webb, D. S., Swietzer, S. M., Petricoin, E. F. & Finbloom, D. S. (1993) *Science* **261**, 1730–1733
27. Ruff-Jamison, S., Chen, K. & Cohen, S. (1993) *Science* **261**, 1733–1736
28. Shuai, K., Schindler, C., Prezioso, V. R. & Darnell, J. E. (1993) *Science* **258**, 1808–1812
29. Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. Y., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N. & Yancopoulos, G. D. (1994) *Science* **263**, 92–95
30. Igarashi, K., Garotta, G., Ozmen, L., Ziemiecki, A., Wilks, A. F., Harpur, A. G., Larner, A. C. & Finbloom, D. S. (1994) *J. Biol. Chem.* **269**, 14333–14336
31. Zhong, Z., Wen, Z. & Darnell, J. E. (1994) *Science* **264**, 95–98
32. Wegenka, M., Lütticken, C., Buschmann, J., Yuan, J., Lottspiech, F., Müller-Esterl, W., Schindler, C., Roeb, E., Heinrich, P. C. & Horn, F. (1994) *Mol. Cell. Biol.* **14**, 3186–3196
33. Akira, S., Nishio, Y., Inoue, M., Wang, X.-J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M. & Kishimoto, T. (1994) *Cell* **77**, 63–71
34. Yamamoto, K., Quelle, F., Thierfelder, F., Kreider, B., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., Silvennoinen, O. & Ihle, J. N. (1994) *Mol. Cell. Biol.* **14**, 4342–4349
35. Hou, J., Schindler, U., Henzel, W. J., Ho, T. C., Brasseur, M. & McKnight, S. L. (1994) *Science* **265**, 1701–1709
36. Shuai, K., Horvath, C. M., Tsai Huang, L. H., Qureshi, S. A., Cowburn, D. & Darnell, J. E. (1994) *Cell* **76**, 821–828
37. Meier, V. S. & Groner, B. (1994) *Mol. Cell. Biol.* **14**, 128–137
38. Raught, B., Khursheed, B., Kazansky, A. & Rosen, J. (1994) *Mol. Cell. Biol.* **14**, 914–922
39. Wong, P., Severns, C. W., Guyer, N. B. & Wright, T. M. (1994) *Mol. Cell. Biol.* **14**, 1752–1763
40. Fu, X.-Y., and Zhang, J.-J. (1993) *Cell* **74**, 1135–1145
41. Warren, W. C., Munie, G. E. & Glenn, K. C. (1993) *Mol. Cell. Endocrinol.* **98**, 27–32
42. David, M., Petricoin, E. F., Igarashi, K.-I., Feldman, G. M., Finbloom, D. S. & Larner, A. C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 7174–7178
43. Silva, C., Lu, H., Weber, M. & Thorner, M. (1994) *J. Biol. Chem.* **269**, 27532–27539
44. Fu, X., Kessler, D. D., Veals, S. A., Levy, D. E. & Darnell, J. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 8555–8559