Yin-yang 1 and Glucocorticoid Receptor Participate in the Stat5-mediated Growth Hormone Response of the Serine Protease Inhibitor 2.1 Gene*

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A growth hormone-inducible nuclear factor complex (GHINF), affinity-purified using the growth hormone response element (GHRE) from the promoter of rat serine protease inhibitor 2.1, was found to contain Stat5a and -5b, as well as additional components. The ubiquitous transcription factor yin-yang 1 (YY1) is present in GHINF. An antibody to YY1 inhibited the formation of the GHINF-GHRE complex in an electrophoretic mobility shift assay. Furthermore, Stat5 was co-immunoprecipitated from rat hepatic nuclear extracts with antibodies to YY1. An examination of the GHRE shows that, in addition to two γ-activated sites, it contains a putative YY1 binding site between the two γ-activated sites, overlapping them both. Mutation of this putative YY1 site results in a decrease of GHINF-GHRE complex formation in an electrophoretic mobility shift assay and a corresponding decrease in growth hormone (GH) response in functional assays. The glucocorticoid receptor was also present in GHINF, and Stat5 co-immunoprecipitates with glucocorticoid receptor in hepatic nuclear extracts from rats treated with GH. GH activation of serine protease inhibitor 2.1 requires the unique sequence of the GHRE encompassing the recognition sites of several transcription factors, and the interaction of these factors enhances the assembly of the transcription complex.

The liver is a major target organ for growth hormone (GH) action. Recent advances have led to the elucidation of much of the GH signaling pathway. GH binds to the growth hormone receptor (GHR) on the cell surface, triggering GHR dimerization, activation of Jak2 from the JAK family of tyrosine kinases, and the phosphorylation of specific tyrosine residues on the cytoplasmic domains of the GHR (1–5). In turn, latent cytoplasmic transcription factors, known as signal transducers and activators of transcription (STATs) are recruited to the receptor complex and become tyrosine-phosphorylated (6–11). Subsequent phosphorylation of serine/threonine residues, dimerization, and translocation of these STATs to the nucleus is followed by binding to cognate DNA response elements of target genes, ultimately resulting in their transcriptional induction (12–14).

The serine protease inhibitor (Spi) 2.1 gene has served as a useful model for investigating the mechanism of GH action in rat liver. We delineated a GH response element (GHRE) in the promoter of Spi 2.1 that includes two γ-activated sites (GAS), both of which are necessary for a GH response in functional assays (15). In the hypophysectomized rat, as well as in several cell lines, GH activates Stat1, -3, and/or -5 (7–10, 15–19). Stat5, first purified as the prolactin (PRL)-responsive mammary gland factor from sheep mammary glands (20), has since been purified and cloned from mouse liver and IM-9 lymphocytes (19, 21). We purified a GH-inducible nuclear factor complex, GHINF, from rat liver, in which the major DNA binding component was Stat5 (15). We also found that the GHINF complex contained additional unknown proteins. We now report the identities of the additional components and evidence for their role in the GH induction process.

**EXPERIMENTAL PROCEDURES**

**Treatment of Rats—** All animals were handled in accordance with experimental protocols approved by the University of Minnesota Institutional Committee on the Care and Use of Animals. Male rats (Harlan Sprague Dawley) were hypophysectomized at 100–125 grams by the supplier (Harlan Sprague Dawley, Indianapolis, IN) and observed for 3 weeks to confirm growth failure. Administration of GH (Lilly) to these rats at a dosage of 150 μg/100 g, body weight, led to the accumulation of activated Stat5 in liver nuclei (6). Liver nuclei from normal male rats contain variable amounts of activated Stat5, a consequence of the pulsatile nature of the GH level in the male rat (12, 22). We found that treating a normal rat with GH 1 h before sacrifice consistently led to accumulation of activated Stat5 in liver nuclei. Its phosphorylation status, as determined by supershift assays with antibodies to phosphoserine or phosphothreonine with the GHRE, is identical to that observed when a hypophysectomized rat is treated with GH. We therefore used both GH-treated normal and hypophysectomized rats for these studies.

**Electrophoretic Mobility Shift Assays (EMSAs)—** Preparation of crude rat hepatic nuclear extracts, affinity purification of GHINF, and EMSA have all been reported previously (15). Briefly, in an EMSA reaction, 5 μg of hepatic nuclear extracts or ~2 ng of GHINF were incubated with 20 fmol of the radiolabeled probe of interest in a buffer containing 20 mM HEPES (pH 7.6), 10% glycerol, 2 mM MgCl₂, 5 mM CaCl₂, 0.1 mg/ml bovine serum albumin, 4% Ficoll 400, 1 mM spermidine, 1 mM diethiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1 μg of poly(dI-dC). The final KCl concentration was adjusted to 50 mM. After 30 min of incubation at 30 °C, the binding mixture was loaded on to a nondenaturing, 3% glycerol, 5% polyacrylamide gel in a Bio-Rad Protean II system in 0.5× TBE (45 mM Tris, 45 mM boric acid, and 1 mM EDTA, pH 8.3) and electrophoresed at 150 V for 2.5 h. The gel was dried and exposed to film. Quantitation of the shifted complexes on the film was determined using the Kodak Digital Science ID Image Analysis System.
The names of the corresponding plasmid constructs of the oligonucleotides are given in parentheses. The GAS locations in the oligonucleotides are shown in boldface type. Mutations are shown in lowercase type. The glucocorticoid response element in wild type GHRE sequence is shown in superscript. YY1 recognition or mutated sites in all oligonucleotides are underlined.

### Table I

| Name       | Sequence                                                                 | Description                  |
|------------|--------------------------------------------------------------------------|------------------------------|
| YY1        | GATCCACCCCAAATATGGGACGAGATC                                               | Consensus sequence           |
| GHRE       | TCGACCTTCTGACATTCAACATGAATTCAGTGAGTAAATCATCGAGGTCGCCAC                  | Wild type                    |
| (Spi-A-CAT) | TCGACCTTCTGACATTCAACATGAATTCAGTGAGTAAATCATCGAGGTCGCCAC                  | 7 additional bp between two GAS sites |
| K (Spi-K-CAT) | CATTCTAGAATTCTGACATGGATAGAGTAAATCATCGAGGTCGCCAC                        | K and CC to aa within YY1 binding site |
| L (Spi-L-CAT) | CATTCTAGAATTCTGACATGGATAGAGTAAATCATCGAGGTCGCCAC                        | 2 bp between two GAS in GHRE |
| M2         | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | 4 bp between two GAS in GHRE |
| M4         | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | YY1 site on bottom strand    |
| P (Spi-P-CAT) | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | TAA to TcA in 5’ GAS        |
| Q (Spi-Q-CAT) | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | Mutation of YY1 site         |
| R (Spi-R-CAT) | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | 5 additional bp between two GAS |
| S          | TCGACGCTTGTACATAATTCTGAGGATAGAGTAAATCATCGAGGTCGCCAC                    | Wild type                    |
| Primer P1  | GTGCCGAGAC                                                               | For GHRE, M2, M4, P, O, R, S |
| Primer P2  | GTGCCTCAGAGATTCAGTACGCC                                                  | For K and L                  |
| Primer P3  | GATCCTGTCG                                                               | For YY1                      |

Software. In supershift assays, GHINF or hepatic nuclear extracts were incubated with the antibody under investigation for 30 min at 4 °C prior to the addition of the radiolabeled probe.

**Oligonucleotides**—The sequences of the consensus YY1 probe, wild type GHRE, and mutant GHREs are shown in Table I. To prepare double-stranded oligonucleotides, primer P1, P2, or P3 was annealed to the appropriate oligonucleotide as stated in Table I. The annealed duplexes were then extended with the Klenow fragment of Escherichia coli DNA polymerase I and radiolabeled dCTP. The products were subsequently purified through Bio-Spin 6 columns (Bio-Rad).

**Antibodies**—The following antibodies were used in either “supershift” or immunoblot assays. Antibody to YY1 (C-20) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was a polyclonal antibody generated against amino acids 395–414 mapping at the carboxyl terminus of human YY1. Antibody to Gr (P-20) (catalogue no. sc-1081X) is a polyclonal antibody generated against amino acids 774–793 mapping at the carboxyl terminus of human GR. Antibody to Stat5a (L-20) (catalogue no. sc-1081X) is a polyclonal antibody generated against amino acids 711–727 at the carboxyl terminus of mouse Stat5a. This antibody is specific for Stat5a and is nonreactive with Stat5b. Stat5b antibody (C-17) (catalogue no. sc-835 or sc-835X) is a polyclonal antibody generated against amino acids 711–727 at the carboxyl terminus of mouse Stat5b. This antibody reacts with both Stat5a and Stat5b. Antibody to phosphoserine (catalogue no. P3430) and antibody to phosphothreonine (catalogue no. P3555) were from Sigma. These two antibodies do not cross-react with each other. Antibody to YY1 and GR Participate in Stat5-mediated GH Action

**Immunoprecipitations and Immunoblots**—Immunoprecipitations were performed with liver nuclear extracts from either hypophysectomized rats or normal rats treated with GH. Aliquots containing 100 µg were incubated with 2 µg of the antibody of interest in radiointroimmunoprecipitation buffer (50 mM Tris-HCl, pH 7.5, 1% Nonidet P-40, 0.1% SDS, 150 mM NaCl, 2 mM EDTA, and 0.1% sodium deoxycholate) containing, in addition, 0.2 mM phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, and 10 mM NaF. The mixtures were incubated for 1 h on end over end rotation at 4 °C (19). Protein A-agarose (120 µg) was then added to each mixture, and end to end mixing was continued for 16 h at 4 °C. The mixtures were centrifuged at 700 × g for 5 min at 4 °C. The resulting pellets were washed four times with radiointroimmunoprecipitation buffer, each time collecting agarose by centrifuging for 5 min at 4 °C. SDS electrophoresis sample buffer (40 µl) was added to each final agarose pellet, and the mixture was boiled for 90 s and centrifuged. Five µl of the resultant supernatant were then loaded onto a 7.5% SDS-polyacrylamide gel. Immunoblotting was performed as reported previously (15).

**Plasmid Constructions and Functional Assays**—The construction of Spi 2.1 (–275/+85) into the HindIII/PstI sites of the parental CAT reporter plasmid pCATAn, designated here as Spi-A-CAT, and that of mutations in place of the wild type GHRE sequence in Spi-A-CAT were described previously (15). Based on our observation that the insertion of the Xho site at –116–117 does not disrupt the GH response, we constructed long PCR primers extending from this site to 15 base pairs (bp) 5’ of the putative YY1 recognition site. Within these primers, we inserted short mutated sequences into or upstream of the putative YY1 recognition site. Primers incorporating mutations R, P, K, L, and Q, listed in Table I, were used to generate PCR products extending from –116 to –275. These were subsequently ligated, together with PCR products extending from –116 to +85, into pCATAn. All mutations were confirmed by TaqFxs dye terminator cycle sequencing (Applied Biosystems, Foster City, CA) performed by the Microchemical Facilities at the University of Minnesota.

Functional assays in primary rat hepatocytes were carried out as described previously (15). Briefly, primary hepatocytes were isolated from male Harlan Sprague Dawley rats (180–240 g) using the collagenase perfusion method and plated on 35-mm culture dishes at a concentration of 1.2 × 10^6 cells/plate. After a 4-h attachment period, transfection was performed using Lipofectin reagent (Life Technologies, Inc.) in modified Williams E medium with 27.5 µm glucose for 12–14 h. 500 µg/ml of Matrigel (Life Technologies) was then added to the medium, and the hepatocytes were cultured for 48 h in the presence or absence of 0.5 µg/ml GH. At the end of 48 h, the cells were harvested and lysed in 1× Reporter lysis buffer (Promega Corp., Madison, WI) for chloram-

![FIG. 1. Effects of the addition of anti-Stat5a (aStat5a) or anti-Stat5b (aStat5b) (a), anti-phosphoserine (opSer) (b), or anti-phosphothreonine (opThr) (c) on GHINF binding. Reactions in a and b were carried out with ~2 ng of affinity-purified GHINF and 20 fmol of radiolabeled GHRE as described under “Experimental Procedures.” Reactions in c were carried out with 5 µg of hepatic nuclear extracts from a normal rat treated with GH.](http://www.jbc.org/content/early/2008/04/15/jbc.M800734200/F1.large.jpg)
phenicol acetyltransferase (CAT) assays. Results of the assays were expressed as percentage conversion of chloramphenicol to its acetylated form, as determined by phosphor screen autoradiography (Molecular Dynamics Corp., Sunnyvale, CA). Each experiment was repeated three times with freshly isolated hepatocytes. At least two different plasmid preparations of each construct were tested in these experiments.

RESULTS

GHINF Contains Serine Phosphorylated Stat5a and Stat5b—The GHINF complex was purified from liver nuclear extracts of GH-treated rats using affinity chromatography on a GHRE sequence (−147/−103) from the Spi 2.1 promoter. The GHINF complex contained two bands with apparent molecular masses of ~93 and ~70 kDa. The ~93-kDa band was previously shown to contain Stat5 that was tyrosine-phosphorylated in response to GH treatment. To determine what forms of Stat5 were present in this band and to further examine their phosphorylation states, we used antibodies to Stat5a, Stat5b, phosphoserine, and phosphothreonine in GHRE binding assays. Fig. 1a demonstrates that the GHINF-GHRE complex can be partially supershifted with an antibody specific to Stat5a. An antibody recognizing both Stat5a and Stat5b (Stat5b antibody) supershifted the GHINF-GHRE complex. In addition to containing phosphorylated tyrosine residues, both Stat5a and Stat5b isoforms are serine-phosphorylated, since a phosphoserine antibody supershifted the GHINF-GHRE complex (Fig. 1b). Similarly, an antibody specific for serine-phosphorylated Stat5a/b supershifted the entire GHINF-GHRE complex (data not shown). In contrast, these isoforms of Stat5 are not threonine-phosphorylated, since a phosphothreonine antibody did not alter the GHINF-GHRE complex (Fig. 1c).

GHINF Contains YY1—Examination of the GHRE sequence reveals a potential YY1 binding site, ATCCATGGT, located between the two GAS and overlapping them both (Table I). A comparison of this sequence with the consensus sequence for YY1 binding, (C/g/a)(G/t)(C/t/a)CATN(T/a)(T/g/c), with the uppercase letters denoting preferred bases (23), indicates that this is a lower affinity YY1 binding site. Previously, we showed that YY1 binding, (C/g/a)(G/t)(C/t/a)CATN(T/a)(T/g/c), with the uppercase letters denoting preferred bases (23), indicates that this is a lower affinity YY1 binding site. Previously, we showed that YY1 binds synergistically to the two GAS in GHRE (15). One hypothesis, given the above data, is that YY1 may play a role in Stat5 during the response to GH.

Effects of Mutations of GHRE on GHINF Binding—GHINF binds synergistically to the two GAS in GHRE (15). One hypothesis, given the above data, is that YY1 may play a role in Stat5 during the response to GH.
from the native spacing of 6 bp to 2 bp (M2) or 4 bp (M4) (lanes 1 and 2). Within the wild type spacing of 6 bp, mutating only 2 bp critical to YY1 binding (CC → AA) led to a significant decrease in GHINF binding (R, lane 4), when compared with the wild type GHRE (lane 3). Placement of the putative YY1 binding site on the opposite strand (P) led to little change in GHINF binding (lane 5) when the differences in specific activities of the probes were taken into consideration (Table II). However, increasing the spacing between the two GAS to 11 or 13 bp, thus changing the phase of the GAS (S, K, and L), led to noticeable decreases in the intensities of GHINF binding. Mutation of the TAA of the 5′ GAS to TCA (Q) led to a further decrease in GHINF binding (lane 9). The decreases in GHINF binding to these probes were particularly noteworthy, since the specific activities of these probes were at least 2-fold higher than that of GHRE (Table II). Therefore, either mutating the putative YY1 binding site or changing the phase of the GAS led to significant decreases in GHINF binding. Thus, the synergistic binding of two dimers of Stat5, the length of spacing between these two Stat5 binding sites, and the sequence of that spacing are all critical factors in the ability of GHRE to respond to GH.

GHINF Contains GR—Stat5 can associate with GR when both are overexpressed in COS7 cells (25). In addition, a role for GR in the Stat5-mediated PRL induction of the β-casein gene has been reported (26). To determine if GHINF purified from livers of rats after GH treatment also contains GR, we used an antibody to GR to probe an immunoblot of purified GHINF (Fig. 6a). A ∼93-kDa band that is immunoreactive with GR antibody was detected in purified GHINF. Of note, GR and Stat5 have similar molecular masses and migrate to similar positions on SDS-polyacrylamide gel electrophoresis (26). To assess whether GR associates with Stat5 during a GH response, we carried out GR antibody co-immunoprecipitations of hepatic nuclear extracts from hypophysectomized rats and normal rats treated with GH. Fig. 6b shows an immunoblot of these immunoprecipitates probed with an antibody to Stat5b. In the immunoprecipitate from the hepatic nuclear extract from a normal rat treated with GH, there is a band migrating to ∼93 kDa that is immunoreactive with Stat5b antibody (lane 2). This band is absent in liver nuclear extracts from hypophysectomized rats (lane 1). Similar results were obtained when Stat5 antibody immunoprecipitates were probed with GR antibody (not shown). Thus, GR is associated with Stat5 during a GH response.

**DISCUSSION**

Previously, we have shown that the affinity-purified GHINF complex contains Stat5 and that it is tyrosine-phosphorylated (15). We now show that both isoforms of Stat5, Stat5a and Stat5b, are present in GHINF and that they are, in addition to being tyrosine-phosphorylated, serine-phosphorylated. Tyrosine and serine residues of Stat5 are sequentially phosphorylated during a GH response (14). While tyrosine phosphorylation is necessary for Stat5 binding to GHRE, the role of serine phosphorylation is unknown. Stat5a mRNA is found predominantly in mammary tissues during lactation, whereas Stat5b message is found at similar levels in both liver and mammary tissues (21).

Stat5 binds synergistically to two GAS in GHRE from the Spi 2.1 promoter, and the migration of the Stat5/GHRE complex is more retarded than that of a Stat5 dimer bound to a single GAS (15), consistent with a higher order complex composed of two interacting STAT dimers (27). Similar cooperative binding to neighboring sites has been reported for Stat1, -4, and -6 (28). Many STAT proteins bind weakly, or not at all, to individual native sites. Interactions between dimeric molecules bound on
The relative relationships of activities within struct's activity to that of Spi-A-CAT was calculated as percentage conversion of chloramphenicol to its acetylated forms. The values shown are representative of four separate experiments. In each experiment, the ratio of each mutation construct’s activity to that of Spi-A-CAT was within ±10% of that shown in this figure. The relative relationships of activities shown in this figure were seen for all experiments.

An identical mutation in a lengthened spacing between the two GAS (Spi-L-CAT) led to an almost total abrogation of the GH response. These results indicate that YY1 or another factor that recognizes this sequence promotes the synergistic binding of Stat5 to the two GAS in GHRE, an event that is necessary for Spi 2.1 to respond to GH. The observation that both Spi-R-CAT and Spi-L-CAT, containing only a mutation of 2 bp critical to YY1 binding (CC → AA), responded weakly to GH strongly suggests that YY1 is the factor involved. Taken together, these results suggest that YY1 enhances the GH response of Spi 2.1 by promoting the assembly of an active conformation of the Stat5 tetramer-GHRE complex.

YY1 is a ubiquitously expressed, zinc finger-containing factor with homology to the GLI-Krüppel family of proteins (30). It participates in different processes associated with gene transcription, including both activation and repression. It binds to, among others, TAFII55-TATA-binding protein and cAMP-response element-binding protein-binding protein (31). The sequences flanking either side of the YY1 core “CAT” binding site can be quite variable, presenting opportunities for its site to overlap with other DNA binding sites. For example, YY1 and the serum response factor binding sites overlap in the c-fos promoter (32). Competition between these factors has been proposed as one of the mechanisms by which YY1 represses transcription (30, 33). In the case of β-casein, where the YY1 site overlaps a GAS in the prolactin response element, PRL acts by overcoming the repression of YY1 (34, 35). In other cases, YY1 mediates transcription by binding the DNA to promote contacts between other proteins that interact within the promoter and enhancer domains (33, 36, 37). We now show that YY1, in sharing overlapping sites with Stat5 at each end of its binding site in GHRE, acts by enhancing transcription of Spi 2.1 during a GH response. We suggest that it does so by facilitating or stabilizing the formation of an active complex of the two Stat5 dimers bound to adjacent GAS.

Given the significance of the YY1 recognition site in the formation of the Stat5 tetramer complex in EMSA and in Spi 2.1 functional assays, it is puzzling that YY1 binding to GHRE is not demonstrable in EMSA. We have previously performed cold competitions with unlabeled YY1 on GHINF binding. The addition of a 25-fold molar excess of unlabeled YY1 did not affect GHINF binding; nor did it affect the ability of the antibody of YY1 to ablate GHINF binding. In hepatic nuclear extracts, the addition of a 25-fold molar excess of unlabeled
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GHRE did not affect YY1 binding. However, not all binding of transcription factors to their cognate sequences on target genes is demonstrable in EMSA. For example, in acute-phase reactions, the presence of Stat3 is usually confirmed by its binding to a high affinity, mutated six-inducible element but not as easily to the wild type sequences of lower affinity that occur in the native promoters of acute-phase reactants (18). Similarly, we show that it is possible to enhance YY1 binding to GHRE in EMSA by placing an additional G or C in the immediate 5'-flanking region of the 9-bp YY1 recognition site (Q and K).

The possibility exists that the involvement of YY1 with Stat5 occurs primarily via a protein/protein interaction and that its actual binding to DNA within the GHRE plays only a secondary role. Immunoprecipitation studies of rat hepatic nuclear extracts with YY1 antibody show that YY1 is associated with Stat5 during GH stimulation. However, functional studies of mutation of the putative YY1 binding site (Spi-R-CAT) indicate that the YY1 recognition sequence is necessary to achieve a level of GH response equivalent to that of the wild type. Therefore, YY1 binding to DNA most likely occurs during the formation of the GHINF-GHRE complex. YY1 exhibits very rapid on/off rates, less than 10 s in vitro (25), and several examples of it serving as an initiator binding protein during the formation of the basal transcription complex have been reported (38–40). Hence, YY1 binding to the GHRE may occur as a transient interaction during the process of Stat5 binding.

The role of YY1 in the GH activation of Spi 2.1 in the liver thus appears to be different from its role in the PRL activation of β-casein in the mammary gland. In the case of β-casein, YY1 represses the expression of β-casein in the absence of PRL, and mutation of its recognition site led to hormone-independent induction. Thus, PRL acts by relieving the repression of YY1 (34, 35, 41). For Spi 2.1 expression, however, mutation of the YY1 site (Spi-R-CAT) did not result in hormone-independent induction. Instead, it led to a significant attenuation of the GH response in the entire range of GH concentration tested. The differences in the placement of the YY1 recognition sites in the prolactin response element and GHRE may explain the variations in their functional significance. In the prolactin response element, the YY1 recognition site is 5' of both GAS sequences, and the 3' GAS alone can support the PRL response of β-casein promoter in functional assays (25). In the GHRE, however, the YY1 site occurs between the two GAS and overlaps them both. Moreover, both GAS are necessary to support the Spi 2.1 GH response in functional assays. Thus, the unique placement of the YY1 site in GHRE probably leads to YY1-induced cooperation that facilitates interactions among Stat5 dimers and other components of the transcription machinery, thereby enhancing transcriptional activation.

GR has been shown to associate with Stat3 during interleukin-6-mediated cellular responses (42) and with Stat5 during PRL activation of β-casein expression (25). In our previous studies in the intact rat, we found that Spi 2.1 gene expression is greatly reduced by hypophysectomy and can be restored to only 40% of its normal level by the administration of GH alone. Full restoration requires the synergistic action of GH, thyroxine, corticosterone, and dihydrotestosterone (43). Furthermore, in primary hepatocyte cultures, the GH response of Spi 2.1 is highly dependent on the presence of dexamethasone in the culture medium (44). Therefore, it is not surprising that we now find that GR is associated with Stat5 in the liver, both by its presence in purified GHINF and by its co-immunoprecipitation with Stat5 from hepatic nuclear extracts of normal rats treated with GH. There are several half-glucocorticoid response elements in the Spi 2.1 promoter (45). One of them occurs within GHRE and is situated between the two GAS, overlapping the 3' GAS (Table I). It is not known whether GR actually binds to this site, since the addition of GR antibody in EMSA studies of GHINF binding did not lead to the formation of a supershifted complex, nor did it prevent the formation of the GHINF-GHRE complex (data not shown). Recently, it has been shown that during PRL stimulation, GR acts as a ligand-dependent coactivator that is independent of the specific DNA-binding domain of GR (26). Together with Stat5, GR forms a molecular complex that cooperates in the induction of transcription of the β-casein gene. Specific DNA binding function of the GR is apparently not required for this cooperation, since GR variants that lack active ligand binding domains are able to cooperate with Stat5 in the induction of a β-casein-luciferase construct in COS cells (25). Moreover, GR antibody was able to immuno precipitate a Stat5-GR complex from nuclear extracts that had been incubated with a radiolabeled wild type Stat5-

DNA binding site. No complex was precipitated when a radio labeled mutated Stat5 binding site was used (46). Our results therefore are consistent with the concept that the involvement of GR with Stat5 is probably one of protein/protein interaction that does not depend upon its binding to a glucocorticoid response element.

In addition to Stat5a/b, we have now demonstrated that the GHINF complex also contains YY1 and GR. There is increasing evidence that several activators may act together to facilitate transactivation mediated by a single DNA-binding transcription factor (47). The evidence presented here suggests that, during a GH response, Stat5 becomes both tyrosine- and serine-phosphorylated and probably associates with GR. Following translocation to the nucleus, it also associates with YY1. By virtue of the unique placement of its binding site, we suggest that this sequence of YY1 promotes the assembly of an active conformation of the Stat5 tetramer-GHRE complex. This conformation, interacting with other components of the transcriptional machinery, leads to efficient transcription of Spi 2.1.

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REFERENCES

1. Vanderkuur, J. A., Wang, X. Y., Zhang, L. Y., Allevato, G., Billestrup, N., and Carter-Su, C. (1995) J. Biol. Chem. 270, 21738–21744
2. Yi, W. S., Kim, S. O., Jiang, J., Park, S. H., Kraft, A. S., Waxman, D. J., and Frank, S. J. (1996) Mol. Endocrinol. 10, 1425–1443
3. Krogados, C. O., Toeng, M. J., Lause, C. D., Billestrup, N., and Carter-Su, C. (1996) J. Biol. Chem. 271, 18088–18094
4. Smit, L. S., Meyer, D. J., Billestrup, N., Norstedt, G., Schwartz, J., and Carter-Su, C. (1996) Mol. Endocrinol. 10, 519–533
5. Huisman, M. A., Hansen, L. H., Kato, S., Mailleux, F. J., Guilleux, F., Groner, B., Nielsen, J. H., Moldrup, A., Galgaard, E. D., and Billestrup, N. (1997) J. Mol. Endocrinol. 18, 213–221
6. Berry, S. A., Bergad, P. L., Whaley, C. D., and Towlie, H. C. (1994) Mol. Endocrinol. 8, 1714–1719
7. Gronowski, A. M., Zhong, Z., Wen, Z. L., Thomas, M. J., Darnell, J. E., and Rotwein, P. (1995) Mol. Endocrinol. 9, 171–177
8. Wood, T. J., Sliva, D. L., Lohie, P. E., Pinker, T. J., Guilleux, F., Wakao, H., Gustafsson, J. A., Groner, B., Norstedt, G., and Haldosen, L. A. (1995) J. Biol. Chem. 270, 9448–9453
9. Ram, P. A., Park, S. H., Choi, H. K., and Waxman, D. J. (1996) J. Biol. Chem. 271, 5929–5940
10. Smit, L. S., Vanderkuur, J. A., Stimage, A., Han, Y. L., Luo, G. Y., Yulee, L. Y., Schwartz, J., and Carter-Su, C. (1997) Endocrinology 138, 3426–3434
11. Udy, G. B., Towers, R. P., Snell, R. G., Wilkins, R. J., Park, S. H., Ram, P. A., Waxman, D. J., and Davey, H. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7229–7244
12. Waxman, D. J., Ram, P. A., Park, S. H., and Choi, H. K. (1995) J. Biol. Chem. 270, 13262–13270
13. Gronowski, A. M., Lestunff, C., and Rotwein, P. (1996) Endocrinology 132, 558–564
14. Gebert, C. A., Park, S. H., and Waxman, D. J. (1997) Mol. Endocrinol. 11, 400–414
15. Bergad, P. L., Shih, H. H., Towle, H. C., Schwabenegg, S. J., and Berry, S. A. (1995) J. Biol. Chem. 270, 24903–24910
16. Finbloom, D. S., Petricoin, E. F., Hackett, R. H., David, M., Feldman, G. M., Igarashi, K., Fibach, E., Weber, M. J., Thorner, M. O., Silva, C. M., and Larner, A. C. (1994) Mol. Cell. Biol. 14, 2115–2118
17. Meyer, D. J., Campbell, G. S., Cochran, B. H., Argetsinger, L. S., Larner, A. C., Finbloom, D. S., Carter-Su, C., and Schwartz, J. (1994) J. Biol. Chem. 269, 4701–4704
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18. Campbell, G. S., Meyer, D. J., Raz, R., Levy, D. E., Schwartz, J., and Carter-Su, C. (1995) *J. Biol. Chem.* **270**, 3974–3979
19. Silva, C. M., Lu, H. W., and Day, R. N. (1996) *Endocrinology* **10**, 508–518
20. Wako, H., Gouilleux, F., and Groner, B. (1994) *EMBO J.* **13**, 2182–2191
21. Liu, X. W., Robinson, G. W., Gouilleux, F., Groner, B., and Hennighausen, L. (1995) *Proc. Nat. Acad. Sci. U. S. A.* **92**, 8831–8835
22. Jansson, J. O., Eden, S., and Isaksson, O. (1985) *Endocrinology* **6**, 128–150
23. Hyde-DeRuyscher, R., P., Jennings, E., and Shenk, T. (1995) *Nucleic Acids Res.* **23**, 4457–4465
24. Becker, K. G., Jedlicka, P., Templeton, N. S., Liotta, L., and Ozato, K. (1994) *Gene (Amst.)* **150**, 259–266
25. Stoecklin, K., Wisler, M., Moriggl, R., and Groner, B. (1997) *Mol. Cell. Biol.* **17**, 6708–6716
26. Stoecklin, K., Wisler, M., Gouilleux, F., and Groner, B. (1996) *Nature* **383**, 726–728
27. Darnell, J. E. (1997) *Science* **277**, 1630–1635
28. Xu, X., Sun, Y.-L., and Hoey, T. (1996) *Science* **275**, 794–797
29. Vinkemeier, U., Cohen, S. L., Maorefi, I., Chait, B. T., Kuriyan, J., and Darnell, J. E. (1996) *EMBO J.* **15**, 5616–5626
30. Shi, Y., Lee, J. S., and Galvin, K. M. (1997) *Biochim. Biophys. Acta* **1332**, F49–F66
31. Austen, M., Luscher, B., and Luscher-Firzlaff, J. M. (1997) *J. Biol. Chem.* **272**, 1709–1717
32. Martin, K. A., Gualberto, A., Kolman, M. F., Lowry, J., and Walsh, K. (1997) *DNA Cell Biol.* **16**, 653–661
33. Galvin, K. M., and Shi, Y. (1997) *Mol. Cell. Biol.* **17**, 3723–3732
34. Meier, V. S., and Groner, B. (1994) *Mol. Cell. Biol.* **14**, 128–137
35. Schmitt-Ney, M., Doppler, W., Ball, R. K., and Groner, B. (1991) *Nature* **354**, 241–245
36. Seto, E., Shi, Y., and Shenk, T. (1991) *Cell* **66**, 1115–1121
37. Natesan, S., and Gilman, M. Z. (1993) *Genes Dev.* **7**, 2497–2509
38. Basu, A., Park, K., Atchison, M. L., Carter, R. S., and Avadhani, N. G. (1993) *J. Biol. Chem.* **268**, 4188–4196
39. Cella, N., Groner, B., and Hynes, N. E. (1998) *Mol. Cell. Biol.* **18**, 1752–1763
40. Zhang, Z., Jones, S., Haggood, J. S., Fuentes, N. L., and Fuller, G. M. (1997) *J. Biol. Chem.* **272**, 30607–30610
41. Berry, S. A., Manthei, R. D., and Seelig, S. (1986) *Endocrinology* **119**, 2290–2296
42. Shih, H., and Towle, H. (1995) *BioTechniques* **18**, 813–816
43. Basu, A., Park, K., Atchison, M. L., Carter, R. S., and Avadhani, N. G. (1993) *J. Biol. Chem.* **268**, 4188–4196
44. Cella, N., Groner, B., and Hynes, N. E. (1998) *Mol. Cell. Biol.* **18**, 1783–1792
45. Janknecht, R., and Hunter, T. (1996) *Nature* **383**, 22–23
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