The Growth Hormone-binding Protein Is a Location-dependent Cytokine Receptor Transcriptional Enhancer*

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In the rat, a growth hormone-binding protein (GHBP) exists that is derived from the growth hormone (GH) receptor gene by an alternative mRNA splicing mechanism such that the transmembrane and intracellular domains of the GH receptor are replaced by a hydrophilic carboxyl terminus. In isolation, the GHBP is inactive, although it does compete with the receptor for ligand binding in the extracellular space and therefore inhibits the cellular response to GH. The GHBP is also located intracellularly and is translocated to the nucleus upon ligand stimulation. We show here that endogenously produced GHBP, in contrast to exogenous GHBP, was able to enhance the STAT5-mediated transcriptional response to GH. Interestingly, when the GHBP was targeted constitutively to the nucleus by the addition of the nuclear localization sequence of the SV40 large T antigen, greater enhancement of STAT5-mediated transcription was obtained. The transcriptional enhancement did not require GH per se and was not specific to the GH receptor, since similar enhancement of STAT5-mediated transcription by nuclear localized GHBP was obtained with specific ligand stimulation of both prolactin and erythropoietin receptors. Thus, the GHBP exerts divergent effects on STAT5-mediated transcription depending on its cellular location. The use of a soluble cytokine receptor as a location-dependent transcriptional enhancer and the ligand-independent involvement of the extracellular domain of a polypeptide ligand receptor in intracellular signal transduction provide additional novel mechanisms of transcriptional control.

Growth hormone (GH) is the major regulator of postnatal body growth and initiates its biological actions, including the induction of a number of RNA species in mammalian tissues, by interaction with a specific membrane-bound receptor (1, 2). The GH receptor was the first cloned member of the now extensive cytokine receptor superfamily, which includes the receptors for prolactin (PRL), erythropoietin (EPO), granulocyte colony-stimulating factor, granulocyte-macrophage colony stimulating factor, ciliary neutrophic factor, thrombopoietin, leptin, cardiotrophin I, and the β-chain of interleukin (IL)-2 through IL-7, IL-9, and IL-11 to IL-13 (3). Most receptors of the cytokine receptor superfamily exist in a soluble and transmembrane form (4–7). The function of the transmembrane forms is well documented and includes signal transduction predominantly but not exclusively through the JAK-STAT pathway, resulting in gene transcription (8, 9). The role of the soluble cytokine receptors, with the notable exception of the soluble forms of the IL-6 and ciliary neurotropitir factor (CNTF) receptors (10, 11), appears confined to ligand sequestration in the extracellular space with a consequent impairment of the cellular response to exogenous ligand (4).

A soluble rat growth hormone-binding protein (GHBP) exists that is derived from the GH receptor gene by an alternative mRNA splicing mechanism such that the transmembrane and intracellular domains of the GH receptor are replaced by a hydrophilic carboxyl-terminal sequence (12). An analogous GHBP exists in other species (13), such as humans, but is derived by proteolytic cleavage of the full-length membrane-bound receptor (14), presumably by the action of specific metalloproteases (15). The GHBP has been demonstrated to compete with the receptor for ligand binding in the extracellular space and has been shown to inhibit the cellular response to GH in vitro (16, 17). In vivo, the GHBP has been demonstrated to increase the biological activity of GH by prolongation of the half-life of plasma GH (18). The GHBP is also located intracellularly (19–21) and has also been prominently localized to the nucleus (20). Other components of the GH signal transduction pathway are also located in the nucleus or translocate to the nucleus upon GH stimulation (21–25). Thus, the GH receptor is subject to ligand-dependent nuclear translocation (21), and constitutively nuclear JAK2 is phosphorylated by exogenous GH stimulation (24). Internalization of the GH receptor has been reported not to be necessary to achieve transcriptional activation by GH (26), and therefore the function of the nuclear localization of components of the GH signal transduction pathway is unknown.

We demonstrate here that nuclear localized GHBP functions as a potent enhancer of STAT5-mediated transcription, not only for GH but also for other members of the cytokine receptor superfamily. Thus, the GHBP exerts opposing effects on STAT5-mediated transcription depending on its extra/intracellular location. The use of a soluble cytokine receptor as a location-dependent transcriptional enhancer and the ligand-independent involvement of the extracellular domain of a
polypeptide ligand receptor in intracellular signal transduction provides additional novel mechanisms of transcriptional control.

EXPERIMENTAL PROCEDURES

Materials—Human growth hormone (hGH) was a generous gift of Novo Nordisk (Singapore). oPRL and rGH were obtained from NIDDK (National Institutes of Health), and mEPO was purchased from Roche Diagnostics. All cell culture medium and supplements (for culture medium) were obtained from Sigma. The luciferase assay system was purchased from Promega (Madison, WI). The ECL kit was obtained from Amersham Biosciences. The GH, PRL, and EPO receptor cDNAs used were as described previously (28). Transfection was performed with DOTAP, poly(dI-dC) and the DNA 3′-end labeling kit were purchased from Roche Diagnostics. Monoclonal antibody against hemagglutinin was obtained from Clontech, monoclonal antisemur against phospho-STAT5A/B were from Upstate Biotechnology, Inc. (Lake Placid, NY), monoclonal antisera against green fluorescent protein (GFP) were from Molecular Probes, Inc. (Eugene, OR), and polyclonal antibody against STAT5B were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). mAb 4.3 and recombinant rat GHBP were generous gifts of Dr. W. R. Baumbach (Monsanto Corp.). The production and characterization of the recombinant rat GHBP has been previously described by us (29).

Generation of Stable Cell Transfectants—BRL cells were stably transfected with the complete rat GH receptor cDNA inserted into an expression vector containing the human cytomegalovirus enhancer and promoter (pCDNA). The characterization and use of these cells has previously been described in detail (28). These cells will be referred to as BRL-GHR1-638 cells.

Cell Culture—BRL cells were grown in DMEM (supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM-glutamine) at 37 °C in 5% CO2. hGH, rGH, oPRL, and recombinant rat GHBP were prepared as a stock solution of 1 mg/ml in distilled water. For treatment of cells, hGH, rGH, oPRL, mEPO, and recombinant rat GHBP were diluted in fresh DMEM serum-free medium and added to the cells after transient transfection. Cells were treated with 100 nm hGH unless otherwise specified. GFP was used at 100 nm. mEPO was used at 10 units/ml.

Construction of Expression Plasmids—The cDNA expression plasmid encoding the wild type GHBP under the control of the metallothionein Ia promoter was as previously described (17). In the XS-GHBP construct, the rat GHBP was PCR-amplified without its NH-terminal signal sequence, and an ATG was introduced in the primer just upstream of where the mature GHBP protein is coded. The nuclear localization sequence (NLS)-GHBP was constructed in a similar way, but a nuclear localization signal from the SV40 large T antigen (PKKKRKV) (29) was added upstream of where the mature GHBP is coded. For GHBP-GFP, wild type GHBP was subcloned into α-terminal enhanced green fluorescent protein vector from Clontech (pEGFP-N3) under the control of a cytomegalovirus IE promoter. For the construction of epitope-tagged GHBP mutants, wild type GHBP and NLS-GHBP with or without a stop codon at the position of amino acid 115 were subcloned into a pcDNA-neo vector with a double hemagglutinin tag at the N terminus. The integrity of the reading frame for the GHBP modifications was confirmed by sequence analysis. The construction of GH receptor cDNA expression plasmids containing a deletion, a deletion of box 1 (297–311), or the individual substitution of proline residues 300, 301, 303, and 305 in box 1 for alanine has been described previously (30).

Transient Transfection and Reporter Assay—BRL and BRL-GHR1-638 cells were cultured to confluence in six-well plates. Transient transfection was performed in serum-free DMEM with DOTAP according to the manufacturer’s instructions. 1 µg of reporter plasmid (SPI-GLE1-CAT) and 1 µg of pSV2-LUC were transfected per well. The control or empty vectors served to normalize the amount of DNA transected. For receptor cDNA transfection into BRL cells, 1 µg of each receptor cDNA was microinjected with 1 µg of pCDNA vector. After a 24 h incubation, the medium was changed to serum-free DMEM containing either the respective hormones or GHBP at the indicated concentrations. After a further 24 h, the cells were washed in PBS and scraped into lysis buffer. The protein content of the samples was normalized, and CAT and luciferase assays were performed as previously described (31). Results were normalized to the level of luciferase to control for transfection efficiency and calculated as the fold stimulation of untransfected (non-hormone-treated) cells.

Confocal Laser-scanning Microscopy—BRL cells were grown on glass coverslips in six-well plates and transiently transfected as described above. Fixation was performed with PBS, pH 7.4, containing 4% paraformaldehyde for 10 min at room temperature. Cells were perme-
enhanced GH-stimulated STAT5-mediated transcription at GH concentrations higher than 100 nM is consistent with antago-
nism of the GH effect at high ligand concentrations (34). Trans-
scriptional response to GH (Fig. 3) but not the native GFP. Thus, the GHBP is subject to
ligand-dependent nuclear translocation indicative of an intra-
cellular/intranuclear function. We therefore focused our atten-
dition on the nuclear GHBP.

Cytoplasmic GHBP Enhances GH-stimulated STAT5-mediated Transcription—To determine whether nonsecreted cyto-
plasmically localized GHBP would enhance GH-stimulated
STAT5-mediated transcription, we removed the secretion se-
cuence from the GHBP cDNA (XS-GHBP) (12). In the XS-
GHBP construct, the rat GHBP was PCR-amplified without its
signaling peptide, and an ATG was introduced in the primer
just upstream of where the mature GHBP protein is coded.
XS-GHBP is expressed throughout the cytoplasm of the cell as
observed by confocal laser-scanning microscopy and is not se-
creted to the extracellular space (Fig. 2). Transient transfection
of XS-GHBP cDNA also increased the STAT5-mediated tran-
scriptional response to GH. Transfection of increasing amounts
of XS-GHBP cDNA further enhanced GH-stimulated STAT5-
mediated transcription. As observed with WT-GHBP, transient
transfection of XS-GHBP cDNA exerted no significant effect on
STAT5-mediated transcription in the absence of concomit-
ant transcription of GH receptor cDNA (Fig. 4). Thus, the intracel-
ular GHBP enhances GH-stimulated STAT5-mediated tran-
scription independent of its secretion to the extracellular space.

Ligand-dependent Nuclear Translocation of the GHBP—It has been previously reported that the GHBP is located intra-
cellularly in both the cytoplasm and the nucleus in both an
insoluble form attached to membranes and a soluble form in
the cytoplasm or nucleoplasm. To determine whether the
GHBP was subject to ligand-dependent nuclear translocation,
we subcloned the WT-GHBP into a N-terminal enhanced fluo-
rescent protein vector. The WT-GHBP was therefore expressed
as a fusion protein to the N terminus of the enhanced GFP. The
integrity of the reading frame was confirmed by sequence anal-
ysis, and protein expression was examined by Western blot
analysis and confocal laser-scanning microscopy (Fig. 5A). The
WT-GHBP-GFP was expressed as a protein with a molecular
mass of 67 kDa in contrast to the native GFP, which was
expressed as a protein of 27 kDa. Examination of the cellular
distribution of the WT-GHBP-GFP demonstrated distinct per-

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FIG. 1. Effect of exogenous recombinant rat GHBP on GH stim-
ulation of STAT5-mediated transcription in BRL-GHR<sub>1</sub>-<sub>638</sub> cells. BRL-GHR<sub>1</sub>-<sub>638</sub> cells were cultured to confluence and transiently trans-
ected with SPT-GLE1-CAT as described under “Experimental Proce-
dures.” Cells were treated for 24 h with 1 nM hGH in the presence of the
indicated concentrations of recombinant rat GHBP. Results are pre-
presented as the mean ± S.E. of triplicate determinations of the -fold
stimulation above non-hormone-stimulated cells.

FIG. 2. Expression in BRL cells of transiently transfected wild
type GHBP (WT-GHBP), a GHBP with the amino-terminal secre-
tion sequence removed (XS-GHBP), and a GHBP with the ami-
no-terminal secretion sequence replaced by the nuclear local-
ization sequence of SV40 large T antigen (NLS-GHBP). A, schematic diagram of the WT-GHBP, XS-GHBP, and NLS-GHBP pro-
teins encoded by their respective cDNAs. B–E, localization of the ex-
pressed proteins in BRL cells by immunofluorescence with the empty
vector (B) as a control. WT-GHBP is expressed in the perinuclear region
of the cell (C), XS-GHBP is expressed in the cytoplasm (D), and NLS-
GHBP is expressed in the nucleus. mAb 4.3 directed against the hydro-
philic C terminus of the GHBP was used for detection. F, Western blot
analysis of medium from BRL cells transiently transfected with WT-
GHBP, XS-GHBP, and NLS-GHBP cDNAs.
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Fig. 3. A, effect of transient transfection of WT-GHBP cDNA on the STAT5-mediated transcriptional response to GH in BRL cells transiently transfected with GHR cDNA. BRL cells were cultured to confluence and transiently transfected with GHR cDNA, SPI-GLE1-CAT, and the indicated amounts of GHBP cDNA. Cells were treated for 24 h with 50 nM GH. C, control. Raw data for the cells transfected with 1 μg of either vector or WT-GHBP cDNA are as follows: nonstimulated, 585 ± 42 and 554 ± 57, respectively; stimulated, 1778 ± 351 and 2886 ± 193, respectively. B, effect of increasing concentrations of GH on the STAT5-mediated transcriptional response to GH in the presence of transiently transfected vector and WT-GHBP cDNA. BRL cells were cultured to confluence and transiently transfected with GHR cDNA, SPI-GLE1-CAT, and 1 μg of WT-GHBP cDNA. Cells were treated for 24 h with the indicated concentrations of GH. Results are presented as the mean ± S.E. of triplicate determinations of the -fold stimulation above non-hormone-stimulated cells.

Effect of Nuclear Localized GHBP on GH-stimulated STAT5-mediated Transcription—To examine the function of the nuclear localized GHBP, we introduced the NLS of the SV40 large T antigen at the N terminus of the GHBP (NLS-GHBP). The NLS-GHBP was constructed by replacement of the secretion signal of the WT-GHBP with the nuclear localization signal from the SV40 large T antigen (PKKKRKV) (29). The integrity of the reading frame for the GHBP modification was confirmed by sequence analysis, and translation and location of the protein product was determined by confocal laser-scanning microscopy (Fig. 2). The NLS-GHBP was predominantly localized to the nucleus and was not secreted from the cell and therefore could be utilized to study the functional effect of nuclear localized GHBP on GH-stimulated STAT5-mediated transcription. Transient transfection of BRL cells with both GH receptor cDNA and NLS-GHBP cDNA resulted in a dramatic enhancement of GH-stimulated STAT5-mediated transcription (Fig. 6A). Transient transfection of BRL-GHR1–294 cells with NLS-GHBP cDNA also resulted in dramatic enhancement of GH-stimulated STAT5-mediated transcription (data not shown).

The transcriptional enhancing effect of NLS-GHBP on GH-stimulated STAT5-mediated transcription was increased with the transfection of increasing amounts of NLS-GHBP cDNA (Fig. 6A). No STAT5-mediated transcriptional response to GH was obtained upon transfection of NLS-GHBP cDNA without concomitant transfection of GH receptor cDNA (Fig. 6A). The ability of NLS-GHBP to enhance GH-stimulated STAT5-mediated transcription was observed over a wide concentration range of both the homologous rat GH (Fig. 6B) and human GH (Fig. 6C). The ability of the nuclear localized GHBP to enhance GH-stimulated transcription was not observed when a STAT1/3-responsive reporter plasmid (c-fos-SIE-CAT) (35) was utilized instead of the STAT5 reporter (SPI-GLE1-CAT) (data not shown). Thus, the transcriptional enhancing effect of the nuclear localized GHBP has some specificity for GH-stimulated STAT5-mediated transcription. Similarly transient transfection of NLS-GHBP cDNA does not alter the activity of reporter plasmids that constitutively express either CAT (pCMV-CAT) or luciferase (pSV2-LUC).

Regions in the Intracellular Domain of the GH Receptor Required for NLS-GHBP to Enhance GH-stimulated STAT5-mediated Transcription—As described above, no STAT5-mediated transcriptional response to GH was obtained upon transfection of NLS-GHBP cDNA without concomitant transfection of GH receptor cDNA. We therefore next examined the regions in the intracellular domain of the GH receptor required for NLS-GHBP to enhance GH-stimulated STAT5-mediated transcription. NLS-GHBP cDNA was co-transfected into BRL cells with the cDNA encoding various receptor mutations or deletions as indicated in Fig. 7. No GH stimulation of STAT5-mediated transcription was observed with GH receptor mutations for NLS-GHBP to enhance GH-stimulated STAT5-mediated transcription. Similarly transient transfection of NLS-GHBP cDNA does not alter the activity of reporter plasmids that constitutively express either CAT (pCMV-CAT) or luciferase (pSV2-LUC).

Regions in the Intracellular Domain of the GH Receptor Required for NLS-GHBP to Enhance GH-stimulated STAT5-mediated Transcription—As described above, no STAT5-mediated transcriptional response to GH was obtained upon transfection of NLS-GHBP cDNA without concomitant transfection of GH receptor cDNA. We therefore next examined the regions in the intracellular domain of the GH receptor required for NLS-GHBP to enhance GH-stimulated STAT5-mediated transcription. NLS-GHBP cDNA was co-transfected into BRL cells with the cDNA encoding various receptor mutations or deletions as indicated in Fig. 7. No GH stimulation of STAT5-mediated transcription was observed with GH receptor mutations that lacked the proline-rich box 1 region of the GH receptor (GHR1–294, GHR1–638 Δ297–311, and GHR1–638 P300A, P301A, P303A, P305A) required for association and activation of JAK2 (30, 36) either in the presence or absence of cotransfected NLS-GHBP cDNA. Truncation of the distal intracellular domain of the GH receptor at amino acid residue 454 or 540 diminished the GH stimulation of STAT5-mediated transcription as expected (37) and completely prevented the ability of NLS-GHBP to enhance GH-stimulated STAT5-mediated tran-
scription. Thus, the ability of the nuclear localized GHBP to enhance GH-stimulated STAT5-mediated transcription requires the activation of JAK2 and residues of the intracellular domain of the GH receptor located between amino acids 541 and 638 that are required for binding and full activation of STAT5 (38, 39).

**NLS-GHBP Increases the Rate of GH-stimulated STAT5-mediated Transcription**—To determine the potential molecular mechanisms by which nuclear localized GHBP enhanced GH-stimulated STAT5-mediated transcription, we first examined the effect of the NLS-GHBP on the rate of GH-stimulated STAT5-mediated transcription. As is observed in Fig. 8, the rate of GH-stimulated STAT5-mediated transcription was dramatically increased in the presence of NLS-GHBP. The differential rate of GH-stimulated STAT5-mediated transcription was limited to the first 6 h after cellular stimulation with GH. Thus, nuclear localized GHBP enhances the rate of GH-stimulated STAT5-mediated transcription.

**Effect of NLS-GHBP on Tyrosine Phosphorylation and DNA Binding Activity of STAT5**—We subsequently examined whether the GHBP-enhanced rate of GH-stimulated STAT5-mediated transcription was due to alteration in the phosphorylation state of STAT5. Tyrosine phosphorylation of STAT molecules is requisite for their dimerization, nuclear translocation, and DNA binding (40). We examined both the nuclear translocation of STAT5 and the appearance of tyrosine-phosphorylated STAT5 in the nucleus. Cellular stimulation with GH resulted in the nuclear translocation of STAT5 concordant with the concomitant appearance of tyrosine-phosphorylated STAT5 in the nucleus. The presence of NLS-GHBP did not alter the ability of GH to stimulate either the tyrosine phosphorylation or nuclear translocation of STAT5 (Fig. 9A). Furthermore, the presence of the NLS-GHBP did not alter the rate of removal of tyrosine-phosphorylated STAT5 from the nucleus as observed by the equal reduced amount of tyrosine-phosphorylated STAT5 180 min after stimulation with GH (Fig. 9A) nor after a prolonged period to 8 h (data not shown). We next examined the effect of nuclear localized GHBP on the ability of GH to stimulate binding of STAT5 to its DNA response element. Electrophoretic mobility shift assay with use of the GAS-like element from the SPI 2.1 gene promoter used in reporter assays demonstrated two distinct binding species in response to cellular stimulation with GH. Two of these two SPI-GLE1 binding species have previously been identified as STAT5 (slower migrating) and STAT1 (faster migrating) (41) in BRL-GHR

**NLS-GHBP Enhances STAT5-mediated Transcription Stimulated by Other Members of the Cytokine Receptor Superfamily**—Since the GHBP could enhance STAT5-mediated transcription without bound ligand, we examined whether NLS-GHBP could function as a transcriptional enhancer for other cytokine receptor superfamily members that also utilize STAT5 for transcriptional activation (43). We therefore transiently
transfected either the PRL or EPO receptors into BRL cells (27) and determined the STAT5-mediated transcriptional response in the presence of NLS-GHBP (Fig. 11). Human GH is also a ligand for the PRL receptor (44), and therefore an activation of the PRL receptor and a STAT5-mediated transcriptional response to hGH via the PRL receptor can be expected (27). STAT5-mediated transcription, stimulated specifically through the PRL receptor either with hGH or with ovine PRL, was also enhanced in the presence of NLS-GHBP to a similar extent as

![Fig. 6](image_url)

**Fig. 6.** Effect of transient transfection of NLS-GHBP cDNA on the STAT5-mediated transcriptional response to GH in BRL cells transiently transfected with GHR cDNA. BRL cells were cultured to confluence and transiently transfected with GHR cDNA, SPI-GLE1-CAT, and the indicated amounts of NLS-GHBP cDNA. Cells were treated for 24 h with 50 nM GH. C, control (panel A). Effect of increasing concentrations of hGH (panel B) and rat GH (panel C) on the STAT5-mediated transcriptional response in the presence of transiently transfected vector and NLS-GHBP. BRL cells were cultured to confluence and transiently transfected with GHR cDNA, SPI-GLE1-CAT, and 5 μg of NLS-GHBP cDNA. Cells were treated for 24 h with the indicated concentrations of GH. Results are presented as the mean ± S.E. of triplicate determinations of the -fold stimulation above non-hormone-stimulated cells.

Fig. 7. A, regions of the GH receptor required for the STAT5-mediated transcriptional enhancing effect of NLS-GHBP. BRL cells were cultured to confluence and transiently transfected with the cDNA for the respective GHR mutation. Cells were treated for 24 h with 50 nM GH. Results are presented as the mean ± S.E. of triplicate determinations of the -fold stimulation above non-hormone-stimulated cells. B, schematic diagram of the GH receptor mutations encoded by their respective cDNAs.

We have described here a new functional and ligand-independent role for the soluble extracellular domain of the growth hormone receptor otherwise known as the GHBP. Exogenously applied GHBP behaves as expected (16) and inhibits the cellular response to GH in vitro. In contrast, endogenously produced GHBP functions as an enhancer of cytokine receptor-stimulated STAT5-mediated transcription. Such enhancement of cytokine receptor-stimulated STAT5-mediated transcription is mediated predominantly in the nucleus and does not require

**DISCUSSION**
the presence of the ligand per se. The use of a soluble cytokine receptor as a location-dependent transcriptional enhancer and the ligand-independent involvement of the extracellular domain of a polypeptide ligand receptor in intracellular signal transduction provides additional novel mechanisms of transcriptional control.

The intracellular (19–21) and nuclear localization of the GHBP (19, 21) have been reported previously. The localization of the GHBP to the nucleus has been observed both in vivo (19, 21) and in vitro in experimental systems (21). The localization of the GHBP to the nucleus was heterogeneous (19), suggesting that the nuclear localization of the GHBP was dynamic rather than constitutive. We have now demonstrated here that a GHBP-GFP fusion protein translocates to the nucleus upon cellular stimulation with GH or serum. Thus, the GHBP, in addition to secretion to the extracellular space, is also specifically localized to the nucleus. This localization is not constitutive but requires exposure of the cells to a stimulus and is therefore presumably an active process. We had also previously reported that both GH (23) and the GH receptor (21) are subject to a rapid and transient nuclear translocation. At least one function of the nuclear translocation of the growth hormone and its receptor appears to be the phosphorylation of nuclear localized JAK2 (24, 25) (45). Interestingly, however, the nuclear translocation of both GH and the GH receptor are independent of JAK2, suggesting that nuclear translocation of the ligand-receptor/binding protein complexes are distinct from classical JAK-STAT pathways. Whether the GHBP requires ligand for nuclear translocation remains to be determined, as does the mechanism of the nuclear translocation. The characterization of the GHBP-GFP reported here should greatly facilitate delineation of the mechanism of secretion of the GHBP and also translocation of the GHBP to the nucleus.

We have demonstrated here that nuclear localized GHBP functions as an enhancer of STAT5-mediated transcription not only for GH but also for other members of the cytokine receptor superfamily, which utilize STAT5 for transcriptional responses. STAT5 has been demonstrated to be utilized by a number of GHBP-GFP cDNA and the mutant NLS-GHBP1–115 on the STAT5-mediated transcriptional response to GH in BRL cells. A, Western blot analysis of cell extract from BRL-GHR cells transiently transfected with a plasmid containing the hemagglutinin-tagged cDNA for either the WT-GHBP, NLS-GHBP, or NLS-GHBP truncated at amino acid 115. B, transcriptional response to GH in BRL cells transiently transfected with GHBP cDNA and either the WT-GHBP, NLS-GHBP, or NLS-GHBP1–115. BRL cells were cultured to confluence and transiently transfected with GH receptor cDNA and SPI-GLE1-CAT. Cells were treated for 24 h with 50 nM GH. Results are presented as the mean ± S.E. of triplicate determinations of the fold stimulation above non-hormone-stimulated cells.

a Mertani, H., Raccurt, M., Abatte, A., Nilsson, J., Tornell, J., Billestrup, N., Usson, Y., Morel, G., and Lobie, P. E. (2003) Endocrinology, in press.
of the -fold stimulation above non-hormone-stimulated cells.

Results are presented as the mean ± S.E. of triplicate determinations of the fold-stimulation above non-hormone-stimulated cells.

Figure 11. Effect of transient transfection of NLS-GHBP cDNA on the STAT5-mediated transcriptional response to hGH and rGH, oPRL, and mEPO in BRL cells transiently transfected with the GH receptor, PRL receptor, or EPO receptor cDNA, respectively. BRL cells were cultured to confluence and transiently transfected with 50 ng hGH or rGH, 100 ng oPRL, or 10 units/ml mEPO, respectively. GH receptor, PRL receptor, or EPO receptor cDNA, respectively.

The molecular mechanism by which the NLS-GHBP enhances cytokine receptor-stimulated STAT5-mediated transcription remains to be determined. We have observed that the nuclear localized GHBP does not alter GH-stimulated tyrosine phosphorylation, nuclear translocation, or DNA binding of STAT5. We are also unable to detect an association between the GHBP and STAT5.3. Tyrosine phosphorylation, nuclear translocation, and even DNA binding of STAT5 is not sufficient for STAT5 to induce transcriptional activity (55), suggesting that additional factors are involved in the activation of STAT5. Indeed, multiple co-activators and repressors that interact with STAT5 have been identified (56–58).

In conclusion, we demonstrate here that nuclear localized GHBP functions as a potent enhancer of STAT5-mediated transcription, not only for GH but also for other members of the cytokine receptor superfamily. Thus, the GHBP exerts opposing effects on STAT5-mediated transcription depending on its extra-/intracellular location. The use of a soluble cytokine receptor as a location-dependent transcriptional enhancer and

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the ligand-independent involvement of the extracellular domain of a polypeptide ligand receptor in intracellular signal transduction provide additional novel mechanisms of transcriptional control. What remains to be determined is the mechanism by which the nuclear localized GHBP functions as a transcriptional enhancer. Identification of proteins interacting with the GHBP should be useful in this regard, and such experiments are in progress.

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