Role of GHF-1 in the Regulation of the Rat Growth Hormone Gene Promoter by Thyroid Hormone and Retinoic Acid Receptors*

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In non-pituitary HeLa cells the unliganded thyroid hormone or retinoic acid receptors cause a strong activation of the rat growth hormone promoter that is repressed by their ligands. In contrast, after expression of the pituitary-specific transcription factor GHF-1, thyroid hormone and retinoic acid produce a stimulation similar to that found in pituitary cells. Therefore, GHF-1 changes a ligand-dependent inhibition into a ligand-dependent activation. The essential role of GHF-1 on the rat growth hormone promoter was also demonstrated with AF-2-defective T3 receptor mutants that show a normal activation of this promoter in the presence of GHF-1. Furthermore, a truncated T3 receptor, which lacks the N-terminus and the DNA binding domain, was able to stimulate this promoter in the presence of GHF-1 and exogenous RXR receptors, suggesting the importance of protein to protein interactions in this regulation. This study shows that the final transcriptional effect depends not only on the type of regulatory promoter response elements but also on the presence of other transcriptional activators, in the case of the growth hormone promoter, the tissue-specific transcription factor GHF-1, which plays a coactivator-like role in this promoter.

Expression of the rGH gene has been analyzed to understand cell type-specific transcriptional control as well as regulation by nuclear receptors (1). The proximal rGH promoter contains DNA binding sequences for the pituitary-specific transcription factor GHF-1/Pit-1 at nucleotides −65 to −95 and −107 to −137 (2, 3). GHF-1 expression appears to be essential to transactivate the GH gene (4). The rGH gene transcription is strongly stimulated by the thyroid hormone (T3) and by retinoic acid (RA) in pituitary cells (5, 6). The effects of T3 and RA are mediated by nuclear receptors (TRs and RARs) that bind to specific hormone response elements, preferentially as heterodimers with the RXRs (8–10), in the promoter of target genes to activate or repress their transcription (7). The rGH promoter contains a positive TRE/RARE located at −167 to −190 bp upstream of the transcription start site, which appears to mediate the stimulation by T3 (11) and RA (12, 13). The importance of this sequence in vivo is shown by studies with transgenic mice which have demonstrated that the two G HF-1 binding sites are necessary, but not sufficient, for efficient transcriptional activation of the rGH gene promoter. Inclusion of the sequences containing the TRE/RARE in the transgene markedly enhances somatotroph-specific rGH expression suggesting the existence of synergistic interactions between GHF-1 and this element (4). A cooperation between GHF-1 and TR has been confirmed in transient transfection studies (14, 15), and we have recently demonstrated a cooperation between GHF-1 and RAR (16). This cooperation involves direct protein to protein interactions between the RXR/TR and RXR/RAR heterodimers and the pituitary factor (17). In addition to the stimulation of GH promoter constructs containing the positive TRE, T3 has been described to inhibit the activity of constructs containing only the proximal rGH promoter sequences in pituitary cells (18). The inhibitory effect of T3 appears to be mediated by a negative TRE which overlaps the TATA box (19).

Transcriptional regulation by nuclear receptors is achieved through autonomous activation functions (AFs). A constitutive N-terminal AF-1 and a ligand-dependent AF-2 located in the C-terminal region of the ligand binding domain (20, 21). Ligand binding induces a structural modification in helix 12 of the ligand binding domain, which contains the AF-2 (22–24). This change allows the recruitment of coactivator proteins and a ligand-dependent transcriptional activation (25, 26).

Although most TREs mediate repression in the absence of T3 due to receptor binding of nuclear corepressors that are released upon ligand binding (27, 28), depending on the cell type and the nature of the response element, binding of unliganded receptors can also lead to a constitutive activation of gene transcription (29, 30). In this respect it has been reported that transient overexpression of unliganded TR stimulates transcription from rGH promoter constructs in pituitary cell lines (31, 32).

To investigate the contribution of GHF-1 and TR and RAR to the regulation of the rGH promoter in the present study we employed a heterologous cellular system and performed transient cotransfection assays with GHF-1 and wild type and mutant receptors to avoid the problem of endogenous expression in pituitary cell lines. Our results show both ligand-dependent and ligand-independent actions of TR and RAR on the GH promoter and demonstrate that GHF-1 has a unique regulatory potential in T3- and RA-dependent transactivation of this promoter that was not anticipated, suggesting a coactivator-like role of GHF-1 in TR- and RAR-mediated functions.

**EXPERIMENTAL PROCEDURES**

*Plasmids—GH-CAT constructs containing 5′ deletions (−530 and −145) of the rat GH promoter have been obtained from the previously described constructs (33, 34) by restriction with PstI and XhoI and.

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† The abbreviations used are: rGH, rat growth hormone; T3, 3,5,3′-triiodothyronine (thyroid hormone); RA, retinoic acid; TR, thyroid hormone receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; TRE, thyroid hormone response element; RARE, retinoic acid response element; AFs, activation functions; LBD, ligand binding domain; CMV, cytomegalovirus; RSV, Rous sarcoma virus; CAT, chloramphenicol acetyltransferase; LUC, firefly luciferase; βp, base pair(s).
ligation into a pUC8-CAT backbone. The construct −39/12 bp sequence of the rat GH promoter into the poly linker region of the pUC8 vector. In the plasmids −330GH-CAT, −145GH-CAT, and −39GH-CAT, the AP-1 site of the pUC vector was removed by digestion with NdeI and EcoO109. This treatment deletes a 19-bp fragment from the pUC vectors. Deletions were confirmed by sequencing. Reporter constructs containing positive TRE elements, DR-4 TK-CAT and ΔMMTV-TREGH-CAT, have been described previously (13, 35). The reporter plasmids CMV-LUC and −73CDL-LUC (36) contain the cytomegalovirus and collagenase promoters, respectively, fused to the luciferase reporter gene. Expression vectors for RAR, RXR, and TR contain the cDNA sequences of the α form of the human retinoic acid receptor (hRARα), the human RXR α, and the α1 form of chick TR (21, 31, 37). The expression vectors for the mutants chick TRα (E401Q, E401K, C1, K232I) and TR-(120–408) were previously described (21, 31). The expression vector for GHF-1 contains the cDNA sequence of this transcription factor under the control of the Rous sarcoma virus promoter (38).

Cell Culture, Transient Transfections, and CAT Assays—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and plated 24 h prior to transfection into 60-mm dishes (400,000 cells/dish). The cells were then transfected with calcium phosphate with 10 μg of the reporter plasmid (plus 100 ng of a luciferase internal control plasmid). The amounts of expression vectors for the corresponding transcription factors used are shown in the legend of the corresponding figures. The total amount of DNA from each transfection was kept equal by addition of the corresponding empty expression vectors (RSV-0 and pSG5–0). Treatments with 1 μM RA and 100 nM T3 were administered in Dulbecco’s modified Eagle’s medium containing 10% AG1-XS resin-charcoal stripped newborn calf serum. After 48 h, CAT activity was determined and quantified. Each experiment was repeated at least twice with similar relative differences in regulated expression.

Mobility Shift Assays—Gel retardation assays were carried out with highly purified preparations of TR, RAR, and RXR obtained from vaccinia. As probes we used an oligonucleotide corresponding to −83/12 bp of the rat GH promoter (5′-CTCGAGCTAGGCTTTAAAAGGCCCCGATCTCGAGGAG-3′) or to the T3α and RA palindromic responsive element TREpal (5′-AGCTCTAGGTCATGACCTGTA-3′). For the binding assays the purified proteins were incubated on ice for 15 min in a buffer (20 mM Tris-HCl, pH 7.5, 75 mM KCl, 1 mM dithiothreitol, 5 μg/ml bovine serum albumin, 13% glycerol) containing 1.5 μg of poly(dI-dC) and then for 20–30 min at room temperature with approximately 70,000 cpm of double-stranded oligonucleotide end-labeled with [γ-32P]ATP using T4-polynucleotide kinase. For competition experiments the excess of unlabeled double-stranded oligonucleotides indicated in the figure was added to the labeled DNA. Nuclear protein complexes were resolved on 5% polyacrylamide gels in 0.5 × TBE buffer. The gels were then dried and autoradiographed.

RESULTS

Stimulation of the rGH Promoter by TR in HeLa Cells; T3-Independent and T3-Dependent Components of Synergistic Interactions between GHF-1 and TR—Although expression of the GH gene is restricted to pituitary cells, a measure of promoter activity was routinely obtained in non-pituitary HeLa cells. When the plasmid −530GH-CAT was transfected along with an expression vector for TRα, a strong ligand-independent stimulation of transcription was observed (Fig. 1A, left panel). This activation was similar to that induced by the expression of GHF-1. Unexpectedly, both in the presence and absence of GHF-1, T3 treatment strongly repressed the activity of this construct, which contains the positive TRE located at −190/−167. A constitutive activation by unliganded TR, which was reversed by −150/−180, was displayed by the −145GH-CAT construct (Fig. 1B, left panel), which lacks the TRE, indicating that this element is involved in T3-dependent transactivation and that the putative binding site mediating this effect is located within the first 145 bp of the promoter. Transfection of RXR alone produced no effect in the activity of the rGH fragments tested (data not shown) and had only a slight additive effect in T3-dependent activation of the −530GH-CAT construct, suggesting that endogenous RXR levels in HeLa cells are sufficient for promoter stimulation. Expression of GHF-1 produced an additive stimulatory effect on the constitutive TR-mediated transactivation, and the level of promoter activity achieved by pUC rGH constructs when GHF-1, TR, and RXR were supplied was greater than that produced by each factor alone.

Since the pUC plasmids contain an AP-1-like sequence (39) and liganded TR has been reported to repress AP-1 activity (40), we tested the regulation of plasmids in which the AP-1 binding site has been deleted (hereafter designated with the prefix Δ). The resulting plasmids also displayed a strong ligand-independent activation by TR, but they were only weakly stimulated by GHF-1 (Fig. 1, right panel). In the absence of GHF-1, the −Δ530GH-CAT construct showed a T3-dependent repression of transcription to levels near its basal activity. A most interesting finding was that GHF-1 was able to revert this repression, because in cells cotransfected with TR and GHF-1 T3 induced a strong positive response similar to that observed in pituitary cells, where T3 stimulates GH gene transcription (Fig. 1, right panel). These observations indicate that, indeed, T3 inhibition of the −530GH-CAT construct in cells expressing GHF-1 was mediated by an element of the pUC plasmid backbone. TR requires not only the presence of the GHF-1, but also binding to the positive TRE surrounding the −180 position to mediate T3-dependent stimulation of GH promoter in HeLa cells, because the activity of the shorter construct (−Δ145GH-CAT) was inhibited by T3 both in the presence and absence of cotransfected GHF-1 (Fig. 1B, right panel). Additionally, cooperation between GHF-1 and TR in the absence of ligand was no longer observed in constructs lacking the AP-1 binding site, suggesting that this sequence contributes to the T3-independent interaction between TR and GHF-1.
Fig. 2. GHF-1 is required for T₃-dependent activation of the GH promoter in HeLa cells. The cells were transfected with increasing concentrations of TR and reporter constructs containing the positive TRE (~Δ530GH-CAT) (left panel) or lacking this element (~Δ145GH-CAT) (right panel). The effect of TR expression alone (rectangles) or in combination with GHF-1 (circles) was tested in the absence (open symbols) and in the presence (solid symbols) of 100 nM T₃. Each data point represents the mean values obtained from two independent experiments which did not vary between them more than 5–15%.

Fig. 3 illustrates the effect of varying the amounts of the TR (0.5–4 µg) in the presence and absence of GHF-1 on the activity of the Δ constructs. The constitutive effect of TR was dose-responsive, and the presence of GHF-1 did not increase T₃-independent transactivation at any of the concentrations examined. In all cases, T₃ produced a strong inhibitory effect in the absence of GHF-1. However, in the presence of GHF-1, T₃ treatment caused a strong synergistic stimulation of the ~Δ530GH-CAT plasmid at all TR concentrations (left panel). These results corroborate that TR-GHF-1 synergistic interaction on the activation of GH promoter is dependent on the presence of hormone. As shown in the right panel, T₃ had a repressive effect on the activity of the ~Δ145GH-CAT construct independently of the presence of GHF-1.

Ligand-independent and Ligand-dependent Regulation of GH Promoter Activity by RAR—Fig. 3 shows that when the ~Δ530GH-CAT and ~Δ530GH-CAT constructs were transfected along with an expression vector for RAR, a ligand-independent activation was also observed. Incubation with RA inhibited the activity of the ~Δ530GH-CAT plasmid both in the absence and presence of GHF-1 (left panel). As compared with TR, RAR was less potent in inducing a constitutive activation of the promoter, and the repressive effect of RA was also weaker than that elicited by T₃. In the construct lacking the AP-1 site, the activation by RAR was further enhanced by RA only when GHF-1 was cotransfected. Therefore, the pituitary-specific transcription factor also appears to be required for ligand-independent stimulation of the GH promoter by RA receptors.

Cis-element Responsible for the Constitutive Activation of the rGH Promoter by TR and RAR—To identify the promoter region that mediates the ligand-independent effect of TR and RAR, we performed transient transfection experiments with a shorter rGH promoter fragment (~Δ39GH-CAT). Fig. 4 shows that the increased CAT activity of the ~Δ530GH-CAT construct observed in TR- and RAR-transfected cells was again observed with this shorter construct. In addition, inhibition of reporter gene expression by the liganded receptors was also consistently observed. Fig. 4C demonstrates that a construct containing a consensus TATA sequence is not affected by TR or RAR coexpression. These data suggest that ligand-independent stimulation of the rGH promoter could be mediated by the previously described negative TRE which overlaps the TATA box (19).

Fig. 5A shows in vitro binding of TR, alone or in combination with RXR, to the ~39/+1 rGH promoter fragment. TR bound weakly to this sequence mostly in monomeric form, but heterodimerization with RXR significantly enhanced binding. Similarly, RXR greatly increased binding of RA to this promoter fragment (not illustrated). Panels B and C compare affinity of TR/RXR and RAR/RXR heterodimers for the TATA-associated TRE and for the palindromic TRE (TREpal), a strong binding element for T₃ receptors that also binds RA receptors. Binding of TR/RXR and RAR/RXR to both the ~39/+1 rGH promoter fragment and the TREpal was more efficiently competed by an excess of unlabeled palindromic element than by the TATA-associated element, showing that the latter is a weaker binding element.

GHF-1 Restores the Ligand-dependent Activity of an AF-2-defective TR Mutant—Different mutations in helix 12 of the LBD that have been described to impair ligand-dependent activation (21) were used to analyze the role of the AF-2 domain in the regulation of the rGH promoter by TR. Fig. 6A shows that a TR carrying a point mutation at position 401 (E401Q) did not mediate a substantial repressive effect of T₃ on the ~Δ530GH-AT plasmid in the absence of GHF-1. However, when GHF-1 was cotransfected along with this mutant TR, a normal T₃-dependent transactivation was observed. This unexpected finding suggests that GHF-1 reverts the deleterious effect of this mutation in the interaction with putative coactivators and/or a coactivator-like intermediary role of GHF-1 in rGH promoter regulation. Fig. 6A also shows that a thyroid hormone receptor (C1), carrying the same AF-2 deletion (9 amino acids) as that found in v-erbA, failed to repress
2D
530GH-CAT activity in response to T3. This mutant, as well as another point mutant TR (E401K) (data not shown) also lost the ability to activate ligand-dependent transcription in the presence of GHF-1, probably due to their greatly reduced ligand binding affinity (21). The results obtained with the rGH promoter fragment lacking the positive TRE (\(2D_{145GH-CAT}\)) are shown in Fig. 6B. The wild-type receptor mediated a strong ligand-dependent inhibition of this reporter, and T3 had a diminished ability to repress the activation induced by the unliganded E401Q receptor. These data suggest a putative role of the AF-2 domain in T3-dependent inhibition, because mutations that affect this ligand-dependent activation function also affect T3-dependent repression of the rGH promoter in HeLa cells. Finally, results shown in Fig. 6 show that mutations in the AF-2 domain had no effect on ligand-independent activation by TR.

Other T3-responsive reporters were used to characterize the functional properties of the AF-2 TR mutants in HeLa cells. TR mediated a ligand-independent repression of constructs containing positive TREs (\(2D_{MMTV-TREGH-CAT}\) and \(2D_{DR4-TK-CAT}\)), and this repression was reversed by T3 (Fig. 7, A and B). The unliganded mutant TRs showed a strong repressive effect, but T3-mediated activation was significantly impaired in the E401Q TR and absent in the C1 mutant. Panel C shows the results obtained with the collagenase promoter, which contains an AP-1 binding site, and Panel D shows an unanticipated regulation by TRs of a reporter gene containing sequences of the viral CMV promoter. TR caused a strong ligand-independent activation of both constructs, and T3 repressed reporter gene activity essentially to basal uninduced levels. The AF-2 mutations had parallel effects in T3-dependent transcriptional activation and T3-dependent repression. The E401Q receptor conferred a decreased inhibitory response of the collagenase and CMV promoters to T3, and repression by T3 was totally lost with the C1 receptor. These results show again that the AF-2 region is involved in both responses to T3, activation and repression, but it has no effect on ligand-independent TR actions on the tested promoters.

A deletion mutant of RAR, RAR-(1–390), lacking the last 72 amino acids, which contain the AF-2 region, did not activate the \(2D_{530GH-CAT}\) construct either in absence of RA or in its presence. Furthermore, the truncated RAR exhibited dominant-negative inhibition of RA-dependent activation by the wild-type RAR in the presence of GHF-1 (data not shown).

A Conserved Lysine Residue in Helix 3 Is Involved in Hormone-independent Transactivation of the rGH Promoter—A conserved 20-amino acid region of TR (the \(\pi\) domain) located...
Role of GHF-1 in rGH Promoter Activation by $T_3$ and RA

**DISCUSSION**

**Regulation of rat GH Promoter Activity by Unliganded $T_3$ and RA Receptors in Non-pituitary Cells**—A hormone-independent activation of the rat GH promoter by TR and RAR in HeLa cells, which was reversed after ligand addition has been observed. These results in HeLa cells confirm previous observations showing that overexpression of TR leads to a constitutive activation of the GH and prolactin promoters in a pituitary cell line (31). The sequences involved in constitutive activation mapped to a negative TRE adjacent to the TATA box (19), which we have demonstrated to bind TR/RXR and RAR/RXR heterodimers. Constitutive transactivation by TR of different promoters containing negative hormone response elements has been described previously (29, 30, 41). Although the molecular mechanisms by which the receptors elicit this activation are still unknown, it has been reported recently that the nuclear receptor corepressors activate rather than suppress transcription of genes negatively regulated by thyroid hormone (42).

Besides the AF-2 domain, the $\alpha$ region of the nuclear receptors located in helix 3 has been described as being involved in transactivation (43). Our results show that the AF-2 domain of TR is dispensable for $T_3$-independent transactivation. However, the helix 3 mutation K232I renders a TR unable to induce a ligand-independent activation of the GH promoter in HeLa cells. This is in agreement with the finding that this mutant TR was not able to activate transcription in pituitary cells (32). A mutant estrogen receptor equivalent to TR K232I lacks ligand-dependent transactivation activity, and it has been proposed that this conserved lysine residue, together with residues in helix 12, is required to form the surface by which the receptor interacts with coactivators (44). The finding that this residue is required not only for ligand-induced activity but also for constitutive activation suggest that $T_3$-dependent and -independent transactivation might depend on the same interaction surfaces, although proteins and/or residues interacting might be different.

**Role of GHF-1 in the Response of the GH Promoter to $T_3$ and RA Receptors**—Unexpectedly, in the absence of GHF-1 the activity of GH promoter constructs which contain the positive TRE/RARE surrounding position $-180$ (6, 11) was strongly repressed by $T_3$ in TR-expressing HeLa cells and, less strongly, by RA in RAR-expressing cells. These results suggest that in the absence of GHF-1 the negative response element overlapping the TATA box governs GH promoter activity in HeLa cells. The experiments with mutant TRs show that the C-terminal AF-2 region is required for the $T_3$-dependent repression of the GH promoter obtained under these conditions, since mutation E401Q severely impaired the repressive activity of $T_3$ on the GH promoter.

Interestingly, the ligand-dependent inhibition of the GH promoter obtained in the absence of GHF-1 was transformed into transactivation of the rGH promoter (Fig. 9). In the absence of GHF-1, the truncated receptor showed no transcriptional activity. However, when cotransfected along with GHF-1 and RXR, the truncated TR regained the ability to activate the promoter both in a $T_3$-dependent and -independent manner. Expression of exogenous RXR was required for this response, since $T_3$ (120–408) was inactive when transfected only with GHF-1. A constitutive transactivation, very similar in extent to that mediated by the wild-type TR, was obtained when RXR and GHF-1 were cotransfected. Besides, GHF-1 partially re-stored the $T_3$-dependent activity of the deletion mutant, although this response was weaker than that showed by the wild-type receptor. These results indicate that the ligand binding domain might be a putative region for synergism with GHF-1.

**Fig. 7. Actions of AF-2 mutant thyroid hormone receptors in HeLa cells.** The cells were transfected with expression vectors for the same mutant TRs as in Fig. 6. Effect of a 48-h incubation with 100 nM $T_3$ on the activity of reporter constructs containing the TRE located at positions $-190$/$-167$ of the rGH promoter fused to the murine mammary tumor virus promoter (DMMTV-TREGH-CAT) (A), the consensus TRE sequence in a DR-4 arrangement fused to the TK promoter (DR-4 TK-CAT) (B), a 73-bp fragment of the 5'-flanking region of the collagenase gene containing the AP-1 binding site ($-73$Col-LUC) (C), and the viral CMV promoter (CMV-LUC) (D).

**Within helix 3 of the ligand binding domain has been reported to participate in transactivation of the GH promoter in pituitary cells (32).** Mutation K232I was tested in its ability to transactivate the rGH promoter in HeLa cells. The results obtained are shown in Fig. 6A. Unlike the wild-type receptor, the unliganded mutant TR K232I did not activate the $-\Delta$530GH-CAT construct in the absence of GHF-1. Furthermore, this mutant displayed dominant-negative activity (data not shown). Similar results were obtained with v-erbA. The TR-derived oncoprotein did not activate the promoter in the absence of $T_3$ but inhibited the hormone-independent activity of wild-type TR. Again, GHF-1 played a major role in the activity of the mutant receptor. GHF-1 restored $T_3$-independent transcriptional activity mediated by K232I (but not by v-erbA), since in the presence of the pituitary-specific factor the mutant receptor activated the promoter in a $T_3$-independent manner as effectively as wild-type TR. That this residue also plays an important role in ligand-dependent transactivation is shown by the finding that the response to $T_3$ was significantly reduced in the presence of GHF-1. These results confirm our hypothesis of the putative cofactor role of GHF-1 for rGH transcriptional regulation since its presence, at least partially, repairs the loss of function observed with different TR mutants. Expression of exogenous RXR was required for this response, since $T_3$ (120–408) was inactive when transfected only with GHF-1. A constitutive transactivation, very similar in extent to that mediated by the wild-type TR, was obtained when RXR and GHF-1 were cotransfected. Besides, GHF-1 partially restored the $T_3$-dependent activity of the deletion mutant, although this response was weaker than that showed by the wild-type receptor. These results indicate that the ligand binding domain might be a putative region for synergism with GHF-1.

**GHF-1 Is Able to Confer Ligand-independent and Ligand-dependent Activity to a Mutant TR Lacking the A/B Region and the DNA Binding Domain—A deletion mutant of TR, TR-(120–408), which lacks the first 120 amino acids and cannot bind to DNA, was expressed alone or with RXR to test its ability to...**
a synergistic activation when the receptors were cotransfected along with GHF-1. Therefore, the presence of this factor is essential for T₃ and RA-dependent transactivation of the promoter and reconstitutes the regulation obtained in pituitary cells that express endogenously both the receptors and GHF-1.

Ligand-dependent transactivation of the GH promoter in HeLa cells also requires deletion of an AP-1 element (39) present in the plasmid backbone. Even in the presence of GHF-1, T₃ and RA had a negative effect when the promoter reporter construct contains the AP-1 site. This finding was similar to that obtained with an equivalent estrogen receptor mutant, which conferred ligand-dependent activation to the prolactin promoter in the presence of GHF-1 but was inactive in a consensus promoter (48). In contrast with the results obtained with the E401Q mutation, GHF-1 could not rescue the lack of ligand-dependent activation of a mutant TR lacking 9 amino acids in the AF-2 region or of the E401K mutant. However, the interpretation of these results is unclear because T₃ binding is severely impaired by the deletion and by the Glu→Lys mutation (21). A truncated RAR that lacks 72 C-terminal amino acids was unable to mediate transcriptional stimulation of the GH promoter, but ligand binding affinity is also strongly reduced in this receptor (49). Second, GHF-1 restores the ability of the TR mutant K232I to activate the GH promoter. This receptor was totally unable to induce this activity in the absence of GHF-1. Third, GHF-1 confers ligand-dependent and ligand-independent activity to a truncated TR which contains only the ligand binding domain. This mutant receptor is not only transcriptionally inactive but displays a strong dominant-negative activity on other T₃-responsive promoters in HeLa cells when transfected along with wild-type TR.² Since this receptor cannot bind to DNA, the protein to protein interaction with GHF-1 in vivo might ameliorate binding of the defective heterodimer to the DNA response element.

The results obtained with the E401Q and K232I receptors imply that either the nuclear coactivators are dispensable for the ligand-dependent stimulation of the GH and prolactin promoters, or most likely that GHF-1 induces a conformational change in the mutant receptors, which creates an active interaction surface with coactivators or components of the basal

² T. Palomino and A. Aranda, unpublished observations.
transcriptional apparatus. We have recently observed a direct physical association of T₃ and RA receptors with GHF-1 (17) compatible with this hypothesis. Furthermore, we have recently observed that CBP/p300 cooperates with GHF-1 to stimulate the GH promoter, which may be stabilized by protein-protein interactions. This would favor the formation of complexes containing CBP/p300 and other coactivators with the receptors, which may be stabilized by protein-protein interactions. This would result in the recruitment of these regulatory proteins to the transcription apparatus and in the stimulation of the GH promoter.

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