Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8832–8836, October 1993
Biochemistry

Interaction of human thyroid hormone receptor β with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone

(silencing)

ARIA BANIAHMAD*, ILHO HA†, DANNY REINBERG†, SOPHIA TSAI*, MING-JER TSAI*, AND BERT W. O'MALLEY*‡

*Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; and †Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854

Contributed by Bert W. O'Malley, June 24, 1993

ABSTRACT **The human thyroid hormone receptor β (hTRβ) is capable of both transcriptional silencing and hormone-dependent activation. However, the detailed mechanism of this transcriptional regulation remains to be elucidated. One possibility is that hTRβ interacts directly with factors of the basal transcriptional machinery, thereby modulating basal promoter activity in a direct manner, as has been shown for other transcription factors. Here, we show that hTRβ interacts specifically with the human basal transcription factor TFIIB. Deletion analysis revealed two contact sites in the receptor: one is located in the N terminus, while the other is part of the ligand-binding domain (LBD) and is located at the C terminus. Interestingly, each receptor contact site interacts with different sites in TFIIB. Cotransfection experiments revealed that, when fused to the DNA-binding domain of yeast transcription factor GAL4, the C-terminal interaction site of hTRβ was transcriptionally inactive; however, when it was cotransfected with the remaining part of the LBD on a separate molecule, silencing function was restored. In agreement with that, we show that thyroid hormone is able to significantly decrease the interaction of its receptor LBD with TFIIB. Our data suggest that hTRβ acts as a transcriptional silencer by interacting with TFIIB and that thyroid hormone may act in part by preventing transcriptional repression at this level.**

TFIIB is a basal transcription factor associated with transcription factor TFIID in the transcription initiation complex and is required for transcription of genes by RNA polymerase II. Association of TFIIB with the initiation complex was shown to be the rate-limiting step in gene transcription enhanced by acidic transcriptional activators (1, 2), and TFIIB is known to bind directly to the VP16 acidic activation domain (3). Recently, an interaction between *Drosophila* TFIIB and the glutamine-rich activation domain of the fushi tarazu (*ftz*) gene product was demonstrated in *Drosophila* cells (4), suggesting that TFIIB is a target for transcriptional activators.

The mechanism of transcriptional regulation by steroid/thyroid hormone receptors is largely unknown. Studies from our laboratory with a partially purified fraction containing transcription factor COUP-TF revealed that a cofactor was needed for target gene activation. Subsequently this cofactor was cloned and shown to be TFIIB (5). Cloned COUP-TF I, an orphan receptor and member of the nuclear hormone receptor superfamily, was shown to directly interact with TFIIB. However, transfection experiments showed that COUP-TF I has a transcriptional silencing function similar to that of the human thyroid hormone receptor β (hTRβ) (6). To extend these studies and to analyze the role of hormone in interactions of receptor with basal transcription factors, we have used the well-characterized hTRβ. This receptor is localized in the nucleus and binds to DNA in a hormone-independent manner. The receptor is known to silence transcription in the absence of hormone as well as to possess a hormone-dependent activation function (7). Both functions are localized in the C-terminal part of the receptor.

In this study, we present evidence that hTRβ interacts specifically with human TFIIB. Two parts of the receptor are involved, which bind preferentially to different regions of TFIIB. Cotransfection experiments with parts of the ligand-binding domain (LBD) indicate that both parts of the LBD are required for the silencing function. One of these binds to TFIIB, indicating that TFIIB might be involved in the silencing process. This is supported by the finding that thyroid hormone, which converts the receptor from a silencer to an enhancer, significantly decreases the interaction of the LBD with TFIIB. These data suggest that binding of hTRβ to TFIIB could represent a mechanism for transcriptional silencing and that this interaction can be reversed by ligand.

MATERIALS AND METHODS

Plasmid Construction. For efficient *in vitro* transcription/translation, the full-length cDNAs for chicken β-actin, hTRβ (peA101) (8), and deletion mutants of hTRβ were cloned in the vector pT7βSal (9). The cDNA for human TFIIB and its deletion mutants were cloned in-frame with the glutathione-*S*-transferase (GST)-encoding sequences of the vector pGEX2T (Pharmacia) with a modified multiple cloning site. The expression plasmids pABGAL-TR168-259 and pABGAL-TR260-456 were constructed by insertion of the *Xma* I–*Hin*dII or *Hin*dII–*Hin*dIII fragment, respectively, from pABGAL-hTRβ (7) into the vector pABGAL (10) digested with *Xma* I–*Pvu* II or *Pvu* II–*Hin*dIII.

Transfection Experiments. Cell culture and transfection procedures were described previously (7). In general, transfections were performed using Polybrene (50 μg), the reporter plasmid p17mer 4x tkCAT (10), which contains four copies of the binding site for the yeast transcription factor GAL4 (5 μg), and the expression plasmid (2 μg).

Protein–Protein Interactions. Protein–protein interactions were assayed as described (5, 11) with modifications that will be described elsewhere (C. Baniahmad, A.B., M.-J.T. and B.W.O., unpublished work).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: hTRβ, human thyroid hormone receptor β; LBD, ligand-binding domain; DBD, DNA-binding domain; GST, glutathione *S*-transferase; hsp, heat shock protein; tk, thymidine kinase; CAT, chloramphenicol acetyltransferase.

‡To whom reprint requests should be addressed.

RESULTS

hTRβ Interacts Specifically with Human TFIIB. To demonstrate specific binding of hTRβ to TFIIB, several proteins such as β-galactosidase, human heat shock protein 90 (hsp90), hTRβ, and β-actin were synthesized by translation *in vitro* and labeled with [^{35}S]methionine. A mixture of these proteins was incubated with GST or with GST-TFIIB fusion protein, which were expressed in *Escherichia coli* and immobilized on glutathione coupled to beads. After several stringent washings only hTRβ was retained specifically on the GST-TFIIB beads (Fig. 1*A*), whereas GST alone was unable to retain the receptor. Similar results were obtained with GST-TFIIB protein obtained from a different source, the baculovirus expression system (data not shown). Another set of *in vitro* labeled proteins, including the transcription factor Sp1 and the human heat shock protein 70 (hsp70), were used for additional interaction studies (Fig. 1*B*). Failure to bind to Sp1 demonstrates that binding of hTRβ to TFIIB is not a general phenomenon for transcription factors. Thus, hTRβ is able to bind to TFIIB in a relatively specific manner.

Two Domains of the Receptor Interact with Different Regions of hTFIIB. To localize the domain(s) of interaction within the receptor, various nonoverlapping fragments of hTRβ were generated by selective restriction endonuclease digestion and *in vitro* translation. As shown in Fig. 2, both the N- and the C-terminal parts of the receptor are able to bind specifically to *E. coli*-expressed TFIIB. The N-terminal part is composed of the entire N terminus and the DNA-binding domain (DBD). Binding was still observed after an additional 18 aa had been deleted from the C terminus of this mutant (aa 1–163, Fig. 3*B*). Further deletion from the C terminus decreased the extent of interaction, but the N-terminal 100 aa were still able to bind to TFIIB (data not shown), suggesting

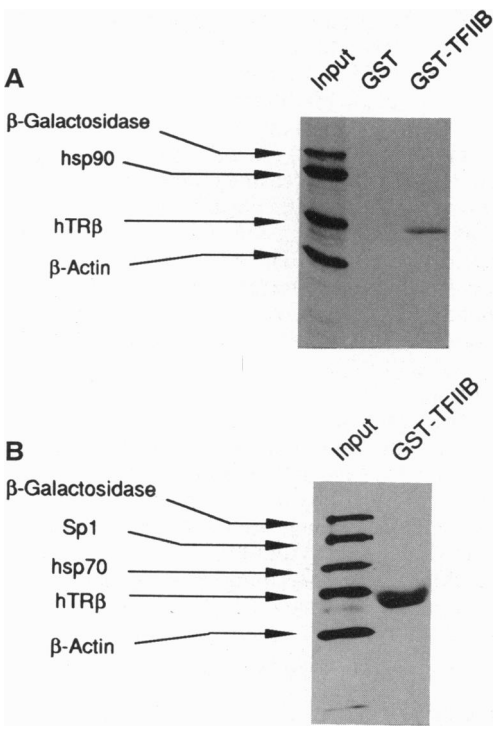

FIG. 1. Specific interaction of hTRβ with *E. coli*-expressed TFIIB. (*A*) A mixture of various *in vitro* translated and labeled proteins was added to GST or GST-TFIIB immobilized on beads. After stringent washing steps the bound proteins were analyzed by SDS/PAGE and visualized by fluorography. (*B*) A different combination of *in vitro* translated and labeled proteins was analyzed for interaction with *E. coli*-expressed GST-TFIIB. Compared with *A*, 5-fold more GST-TFIIB protein was used.

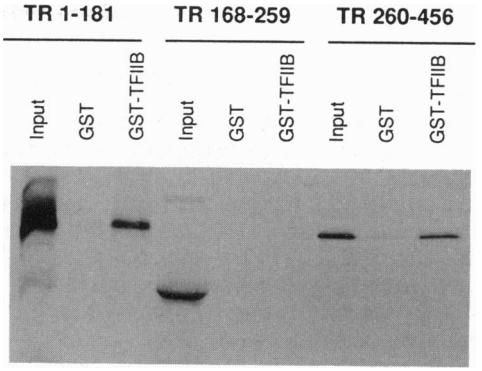

FIG. 2. Two regions of hTRβ interact with TFIIB. Three different regions of hTRβ were labeled by *in vitro* translation and incubated with *E. coli*-expressed GST or GST-TFIIB bound to beads. The numbers indicate amino acid end-points of the truncated receptor. Bound proteins were analyzed by SDS/PAGE as in Fig. 1.

that both the first zinc finger and the N-terminal receptor sequences are required for binding to TFIIB. The second part of the receptor which binds to TFIIB is located in the distal half of the LBD (aa 260–456; Fig. 2). Similar results were seen when baculovirus-expressed GST-TFIIB was used (data not shown). The central part of the receptor containing the

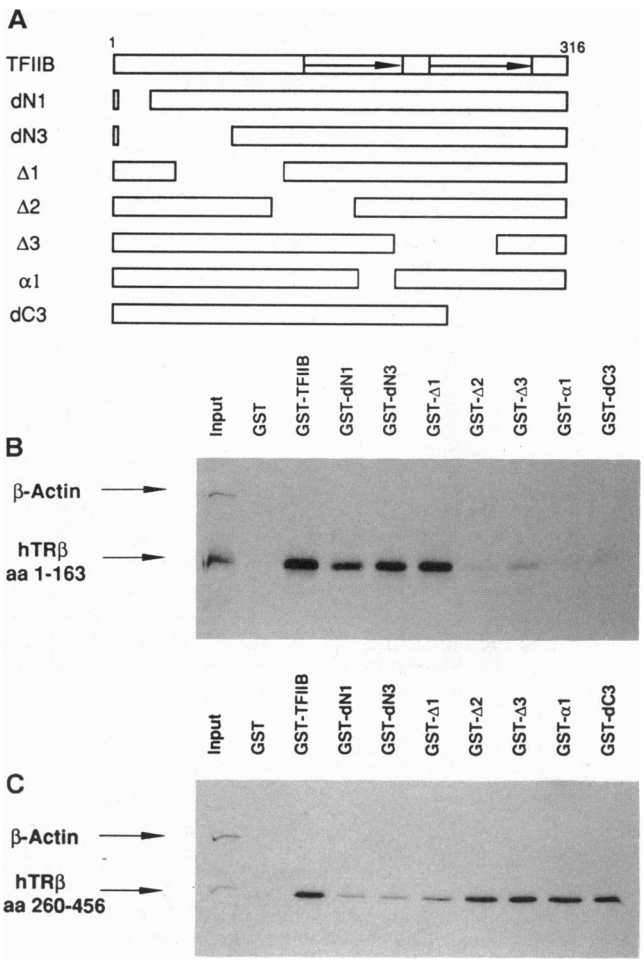

FIG. 3. The N- and C-terminal parts of hTRβ interact differentially with TFIIB. (*A*) Schematic representation of TFIIB and its deletion mutants. The arrows symbolize direct repeats in the TFIIB protein. (*B*) Interaction of the N-terminal part (aa 1–163) of hTRβ with TFIIB in-frame deletion mutants. β-Actin was used as a negative control. (*C*) Interaction of the C-terminal part of the LBD (aa 260–456) of hTRβ with TFIIB in-frame deletion mutants.

hinge region and the proximal part of the LBD (aa 168–259) does not interact with TFIIB.

To localize the regions of TFIIB that are important for interaction with hTRβ, several in-frame deletion mutants (Fig. 3*A*) were tested in a similar approach. Each of the GST-TFIIB deletion mutants was expressed in *E. coli*, and similar amounts were bound to the glutathione-Sepharose beads (data not shown). As shown in Fig. 3*B*, interaction with the N-terminal receptor part was not affected by deletion of the N terminus of TFIIB [TFIIB mutants: dN1 (Δ4–24), dN3 (Δ4–85), and Δ1 (Δ45–123)]. However, interaction with the receptor was greatly decreased in strength with the C-terminal deletion mutants [Δ2 (Δ118–174), Δ3 (Δ178–201), α1 (Δ202–269), and dC3 (Δ238–316)]. These deletion mutants lacked the direct repeats of TFIIB (12). In contrast, the distal part of the LBD interacted strongly with deletion mutants of the C terminus of TFIIB, but binding to TFIIB with N-terminal deletions was significantly impaired (Fig. 3*C*). Thus, two parts of hTRβ, one located in the N terminus and the other in the C terminus of the LBD, interact with different parts of TFIIB.

Silencing Can Be Observed with Two Half-Sites of the hTRβ-LBD Expressed as Separate Proteins. To analyze the functional properties of the receptor fragments which interact with TFIIB, we generated GAL4-TR fusion proteins. The GAL4 DBD (aa 1–147) contains a dimerization domain and a nuclear localization signal and binds specifically to the 17-mer binding site (13, 14), allowing the analysis of truncated receptor fragments. Cotransfection experiments with a reporter containing four copies of the 17-mer sequences in front of a thymidine kinase (tk) promoter–chloramphenicol acetyltransferase (CAT) fusion were performed in L cells. As seen in Fig. 4, in which the obtained data are presented as "fold repression," the GAL4 DBD did not change the promoter activity by itself compared with the control expression vector Δgal (10). Fusion of the entire C terminus (aa 168–456) to GAL4 leads to a hormonally regulated protein with transcriptional properties similar to those of the full-length receptor. Addition of thyroid hormone led to activation, while in the absence of ligand the protein repressed promoter activity due to its silencing function, as was shown previously (7).

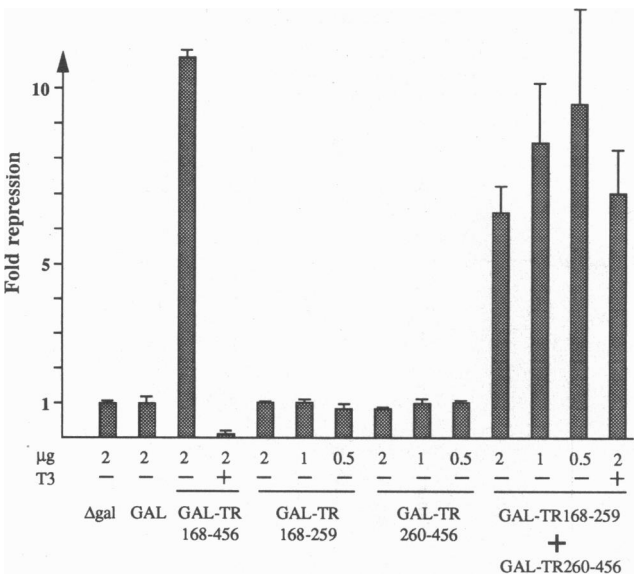

FIG. 4. Silencing is restored by a combined cotransfection of the C- and N-terminal parts of the LBD. Expression plasmids encoding receptor fusions to the GAL4 DBD were cotransfected into L cells with a reporter containing four copies of the GAL4 binding site (17-mer) and the tk-CAT fusion. Basal promoter activity (3.2% conversion of chloramphenicol to acetylated forms or 5000 cpm) was set as 1.00. Fold repression of promoter activity (e.g., 10-fold repression corresponds to production of 500 cpm of acetylated chloramphenicol) is shown in bar graph form with the corresponding standard deviations. The total amounts of expression plasmids used are indicated. Equal amounts of the expression vectors GAL-TR168-259 and GAL-TR260-456 were added. Thyroid hormone (T3) was 0.1 μM when present. Δgal represents an empty expression vector (9).

To define subdomains of the LBD, the entire C terminus of hTRβ was divided into two halves (aa 168–259 and aa 260–456), which were fused separately to the GAL4 DBD and cotransfected independently or in a combination into L cells. These GAL4 fusion proteins did not modulate promoter activity significantly by themselves (Fig. 4), either in the absence or in the presence of thyroid hormone. Similarly, the GAL-TR1-181 fusion had no significant effect on promoter activity (data not shown). However, a combination of the two GAL4 fusions—GAL-TR168-259, which did not interact with TFIIB (Fig. 2), and GAL-TR260-456, which did interact—restored silencing function. Addition of thyroid hormone had no effect on this combination (Fig. 4). Cotransfection of an expression vector encoding TFIIB did not show an effect on the activities specific for hTRβ. Various other combinations, including GAL-TR1-181, did not affect promoter activity either (data not shown).

Thus, silencing can be achieved by dividing the hTRβ C terminus into two subdomains and expressing them together but as different molecules. This suggests that at least two different targets, each interacting with one subdomain, are required to mediate silencing. Since one of the subdomains interacts with TFIIB, this finding indicates that TFIIB may be involved in transcriptional silencing.

The Strength of Interaction with TFIIB Is Decreased by Thyroid Hormone. Since the distal part of the LBD interacts with TFIIB and is involved in silencing, we considered the possibility that binding of hormone to the LBD would interfere with TFIIB interaction. Therefore, the entire C terminus, including the LBD and the hinge region of hTRβ (aa 168–456), was translated and tested for interaction with TFIIB. Similar preferences in binding to the TFIIB mutants observed with TR260-456 (Fig. 3*C*) apply to the entire C terminus (aa 168–456; data not shown). The *in vitro* translated C terminus was then treated with increasing concentrations of hormone. As shown in Fig. 5*A*, at 10^{-8} M thyroid hormone binding to TFIIB was affected and at a concentration of 10^{-7} M, binding was markedly decreased. The dose dependence of this effect is similar to that for reporter activation in cotransfection experiments (data not shown). As controls, a similar molecule, L-tyrosine, or retinoic acid did not affect binding, at levels as high as 10^{-6} M. Similarly, binding of the N terminus of hTRβ (aa 1–163) to TFIIB was not affected by thyroid hormone (Fig. 5*B*). After hormone treatment receptor levels were checked and did not show any significant degradation. Furthermore, we have not observed any influence on the interaction with TFIIB by the presence of retinoid X receptor β and/or the hTRβ binding site DR4 (data not shown). Thus, our results show that thyroid hormone decreases the extent of interaction of the C-terminal part of the receptor with TFIIB.

DISCUSSION

We previously demonstrated that hTRβ represses the basal activity of different minimal promoters containing upstream response elements, due to its silencing function, and activates them in the presence of hormone (7). This suggests that other promoter factors are not required and that the target for regulation by hTRβ is basal promoter factors. Here, we show that hTRβ interacts highly specifically with the basal transcription factor TFIIB *in vitro*. Our data suggest that not all transcription factors interact directly with TFIIB, since bind-

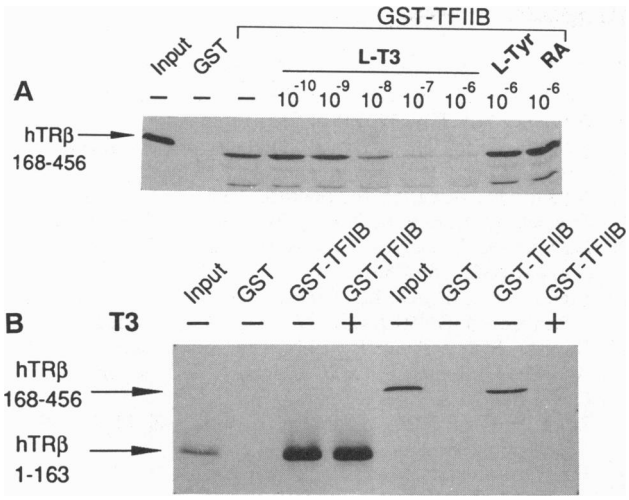

FIG. 5. Thyroid hormone decreases the efficiency of binding of the LBD to TFIIB. (*A*) The complete C terminus of hTRβ (aa 168–456) was translated and labeled *in vitro*. Thyroid hormone (L-T3) at the indicated molar concentrations was incubated with LBD for 20 min at room temperature prior to addition to immobilized TFIIB. Controls contained L-tyrosine (L-Tyr) or retinoic acid (RA). (*B*) The N-terminal sequence of hTRβ (aa 1–163) and the C-terminal sequence (aa 168–456) were incubated with 10^{-6} M thyroid hormone (T3), where indicated, prior to incubation with TFIIB.

ing of Sp1 to TFIIB was not observed. This finding is also in agreement with the observation that promoter activation by Sp1 requires TATA-binding protein-associated factors (TAFs) (15).

It is important that the functional significance of the interactions with TFIIB is elucidated by correlation with the transcriptional properties of the receptor. Both the silencing and activation functions of hTRβ are localized in the C terminus, aa 168–456. No transcriptional function has yet been ascribed to the N terminus, but this region has been shown to contain activation function in many other receptors (16). Two parts of hTRβ bind specifically to different regions of TFIIB. The C-terminal part of the LBD interacts preferentially with the N-terminal region of TFIIB. In contrast to this, the N-terminal interaction site of the receptor recognizes the C-terminal part of TFIIB, which contains the direct repeats and a putative basic amphipathic α-helix. Interestingly, the herpes simplex viral protein VP16 interacts with a similar region of TFIIB (17) as does the N terminus of hTRβ, with the exception that the TFIIB mutant Δ1 (Δ45–123) is interacting with the N terminus of hTRβ but not with VP16. Two point mutants of TFIIB (185/193 and 189/200) were generated, which were unable to bind to VP16. They had little effect on basal transcription but were fully impaired in mediating activation by VP16 (17), suggesting that the putative basic amphipathic α-helix plays an important role in supporting transcription from acidic activators. Recent work with the activation domain of *ftz* suggests that interaction with TFIIB requires the presence of a putative zinc finger in its N terminus (aa 14–35) (4). Therefore, the interaction sites with TFIIB seem to be distinct from those of the VP16 activation domain, suggesting that different transcription factors may activate gene expression through distinct interaction sites of TFIIB.

A conformational change also has been proposed for TFIIB function (4). Conformational changes induced by differential interactions could lead to distinct transcriptional properties of TFIIB. Therefore, it is possible that the C terminus of hTRβ imposes a different function on TFIIB activity than its N terminus, which might indicate that TFIIB is composed of at least two different domains. We decided to focus on the C terminus of the hTRβ and found that the receptor fusion GAL-TR168-259, which did not interact with TFIIB, was transcriptionally inactive by itself. However, when it was cotransfected with the GAL-receptor fusion (aa 260–456), which did interact with TFIIB, strong silencing activity was restored. This suggests that the LBD of hTRβ exerts its silencing function through interaction with TFIIB. In agreement with this, we demonstrated a significant decrease in binding of the LBD to TFIIB upon addition of thyroid hormone. This decrease is probably due to a conformational change in the LBD (22). A possible mechanism for this effect is that in the absence of ligand, hTRβ stabilizes an inactive form of TFIIB in the initiation complex and precludes TFIIB from association with other basal transcription factors. Since the basal transcription factor RAP 30 (TFIIF 30-kDa subunit) is also interacting with the N terminus of TFIIB (23), hTRβ could interfere with that binding and lead to the repression of promoter activity. Hormone blocks the interaction and consequently relieves repression from TFIIB, which is then accessible for activation function by the receptor. We propose that TFIIB is involved not only in mediating activation, but, depending on the nature of its interaction site with regulatory transcription factors, also in mediating other signals, such as silencing.

The detailed role of hormone in receptor activation is largely unknown. A likely mechanism involves relief of inherent repressor function from steroid/thyroid receptors. Different types of repressors may exist. A receptor could have an intrinsic repression function, inhibiting its own activation domains, which is relieved by addition of hormone (18). Also, the cell specificity of the N-terminal activation domain of receptors (16) could be controlled by repressors expressed in a cell-specific manner. Thus, the relief of repressors may represent a general mechanism for steroid/thyroid hormone receptors in gene activation.

It is a distinct possibility that interaction of the receptor with TFIIB is not sufficient to mediate transcriptional regulation. Other basal transcription factors or additional proteins, such as coactivators (bridging factors, mediators), also may be needed for full function. An example of such an interaction with both basal transcription factors and coactivators is given by the viral protein VP16. VP16 interacts with basal transcription factors such as TFIIB (3) and TATA-binding protein (TBP) (19). VP16 point mutants which were deficient for activation were also deficient in binding to TFIIB (3) and TBP (20). In addition, there is evidence for the involvement of coactivators in its transcriptional activation function (21). Similarly, a bridging factor could exist for silencing. The role of the aa 168–259 region of hTRβ in silencing is not yet clear. It could serve as a binding site for a regulatory or bridging factor, which together with TFIIB mediates the silencing function. Taken together, our data suggest that a possible mechanism for transcriptional silencing by hTRβ involves interaction with TFIIB. Furthermore, it seems reasonable that thyroid hormone stimulation of gene expression works in part by releasing the basal transcription apparatus from repression.

We thank Neil F. Rebbe for the human hsp90β cDNA, R. Eisensmith for the β-galactosidase cDNA, B. Wu for human hsp70 cDNA, and R. Tjian for Sp1 cDNA, in vectors suitable for *in vitro* transcription–translation. This work was supported by a fellowship from the Fritz Thyssen Stiftung to A.B. and a grant from the National Institutes of Health (HD08188).

1. Lin, Y.-S. & Green, M. R. (1991) *Cell* **64**, 971–981.
2. Sundseth, R. & Hansen, U. (1992) *J. Biol. Chem.* **267**, 7845–7855.
3. Lin, Y.-S., Ha, I., Maldonado, E., Reinberg, D. & Green, M. R. (1991) *Nature (London)* **353**, 569–571.

4. Colgan, J., Wampler, S. & Manley, J. L. (1993) *Nature (London)* **362**, 549–553.
5. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1992) *J. Biol. Chem.* **267**, 17617–17623.
6. Cooney, A. J., Leng, X., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1993) *J. Biol. Chem.* **268**, 4152–4160.
7. Baniahmad, A., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10633–10637.
8. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M. (1986) *Nature (London)* **324**, 641–646.
9. Norman, C. M., Runswick, M., Pollock, R. & Treisman, R. (1988) *Cell* **55**, 989–1003.
10. Baniahmad, A., Köhne, A. C. & Renkawitz, R. (1992) *EMBO J.* **11**, 1015–1023.
11. Kaelin, W. G., Pallas, D. C., DeCaprio, J. A., Kaye, F. J. & Livingston, D. M. (1991) *Cell* **64**, 521–532.
12. Ha, I., Lane, W. S. & Reinberg, D. (1991) *Nature (London)* **352**, 689–695.
13. Carey, M., Kakidani, H., Leatherwood, J., Mostashari, F. & Ptashne, M. (1989) *J. Mol. Biol.* **209**, 423–432.
14. Silver, P. A., Chiang, A. & Sadler, I. (1988) *Genes Dev.* **2**, 707–717.
15. Pugh, B. F. & Tjian, R. (1990) *Cell* **61**, 1187–1197.
16. Bocquel, M. T., Kumar, V., Stricker, C. & Chambon, P. (1989) *Nucleic Acids Res.* **17**, 2581–2595.
17. Roberts, S. G. E., Ha, I., Maldonado, E., Reinberg, D. & Green, M. R. (1993) *Nature (London)*, in press.
18. Vegeto, E., Allan, G. F., Schrader, W. T., Tsai, M.-J., McDonnell, D. P. & O'Malley, B. W. (1992) *Cell* **69**, 703–713.
19. Stringer, K. F., Ingles, C. J. & Greenblatt, J. (1990) *Nature (London)* **345**, 783–786.
20. Ingles, C. J., Shales, M., Cress, W. D., Triezenberg, S. J. & Greenblatt, J. (1991) *Nature (London)* **351**, 588–590.
21. Berger, S. L., Pina, B., Silverman, N., Marcus, G. A., Agapile, I., Regier, I. L., Triezenberg, S. J. & Guarente, L. (1992) *Cell* **70**, 251–265.
22. Leng, X., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1993) *J. Steroid Biochem. Mol. Biol.* **46**, in press.
23. Ha, I., Roberts, S., Maldonado, E., Sun, X., Kim, L.-U., Green, M. & Reinberg, D. (1993) *Genes Dev.* **7**, 1021–1032.