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GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors

(transcriptional activation)

HENG HONG*, KULWANT KOHLI†, ALPA TRIVEDI‡, DEBORAH L. JOHNSON*‡, AND MICHAEL R. STALLCUP*†§

Departments of †Pathology, *Biochemistry and Molecular Biology, and ‡Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles, CA 90033

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ABSTRACT **The yeast two-hybrid system was used to isolate a clone from a 17-day-old mouse embryo cDNA library that codes for a novel 812-aa long protein fragment, glucocorticoid receptor-interacting protein 1 (GRIP1), that can interact with the hormone binding domain (HBD) of the glucocorticoid receptor. In the yeast two-hybrid system and *in vitro*, GRIP1 interacted with the HBDs of the glucocorticoid, estrogen, and androgen receptors in a hormone-regulated manner. When fused to the DNA binding domain of a heterologous protein, the GRIP1 fragment activated a reporter gene containing a suitable enhancer site in yeast cells and in mammalian cells, indicating that GRIP1 contains a transcriptional activation domain. Overexpression of the GRIP1 fragment in mammalian cells interfered with hormone-regulated expression of mouse mammary tumor virus–chloramphenicol acetyltransferase gene and constitutive expression of cytomegalovirus–β-galactosidase reporter gene, but not constitutive expression from a tRNA gene promoter. This selective squelching activity suggests that GRIP1 can interact with an essential component of the RNA polymerase II transcription machinery. Finally, while a steroid receptor HBD fused with a GAL4 DNA binding domain did not, by itself, activate transcription of a reporter gene in yeast, coexpression of this fusion protein with GRIP1 strongly activated the reporter gene. Thus, in yeast, GRIP1 can serve as a coactivator, potentiating the transactivation functions in steroid receptor HBDs, possibly by acting as a bridge between HBDs of the receptors and the basal transcription machinery.**

Steroid hormone receptors belong to a structurally and functionally related group of intracellular proteins, known as the nuclear receptor or steroid/thyroid hormone receptor superfamily, that serve as ligand-activated transcriptional regulators (1). Binding of the cognate hormone to steroid receptors causes a conformational change that allows the receptors to dissociate from an inhibitory complex of proteins, bind as dimers to specific regulatory sequences (enhancer elements) that are associated with the target genes regulated by the hormone, and modulate the transcription of the target genes. DNA binding by hormone-activated steroid receptors has been shown to cause chromatin remodeling, but the mechanism of transcriptional regulation is also believed to involve some type of direct or indirect interaction of the DNA-bound receptor with the transcription machinery.

Like other nuclear receptors, steroid receptors are composed of three major functional domains: an N-terminal transcriptional activation domain (AD), a central DNA binding domain (DBD), and a C-terminal hormone binding domain (HBD) (1, 2). In spite of this nomenclature, both the N-terminal AD and the HBD contribute to the transcriptional activation function of steroid receptors (3, 4). In the absence of the HBD, the N-terminal AD, called AF-1, can function in a hormone-independent manner in mammalian cells. In contrast, the transcriptional activation function of the HBD, called AF-2, is hormone dependent. Although each isolated AD has some activity in mammalian cells, these two together appear to function synergistically.

The mechanism by which DNA-bound steroid receptors can activate transcription initiation from associated promoters is still unknown. It is proposed that DNA-binding transcriptional activator proteins, including the steroid receptors, stimulate the efficiency of transcription initiation by RNA polymerase II by either directly or indirectly affecting the assembly of basal transcription factors into a preinitiation complex (1, 5). In addition to RNA polymerase II, the preinitiation complex consists of seven basal transcription factors, namely TFIIA, TFIIB, TATA-box binding protein (a subunit of TFIID), TFIIE, TFIIF, TFIIH, and TFIIJ. This complex alone can initiate transcription at a basal rate from TATA-containing promoters, whereas additional TFIID subunits are required for TATA-less promoters and for enhancer-activated transcription. Some of the basal transcription factors may be the targets for regulation by DNA-binding transcriptional activator proteins. Although examples of direct interaction between basal transcription factors and some DNA-binding transcriptional activators have been reported, in most cases the DNA-bound transcriptional activator proteins require coactivators to mediate their effect on the basal transcription machinery (6, 7). By definition, transcriptional coactivators are not required for basal transcription and cannot function as transcriptional activators by themselves because they lack DNA binding domains to tether them to the target gene. Rather, coactivators are required to mediate transcriptional activation by DNA-bound activator proteins, possibly by forming a bridge or by stabilizing a direct interaction, between the activator protein and the basal transcription complex. Coactivators for a few DNA-binding transcriptional activator proteins have been defined; some, called TAFs, are subunits of TFIID (8–10), but others appear to be independent protein components (11–13).

Steroid receptors have been shown to interact physically with basal transcription factors TFIIB (14, 15) and TATA-box binding protein (16) and with TFIID subunit $TAF_{II}30$ (17). While these interactions may contribute to transcriptional activation by steroid receptors, a role for transcriptional co-

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Abbreviations: GRIP, glucocorticoid receptor-interacting protein; HBD, hormone binding domain; AD, transcriptional activation domain; DBD, DNA binding domain; GR, glucocorticoid receptor; ER, estrogen receptor; AR, androgen receptor; DOC, deoxycorticosterone; GST, glutathione *S*-transferase; CAT, chloramphenicol acetyltransferase; βgal, β-galactosidase; CMV, cytomegalovirus.

Data deposition: The GRIP1 sequence reported in this paper has been deposited in the GenBank data base (accession no. U39060).

§To whom reprint requests should be addressed.

activators in mediating steroid receptor function is still an important hypothesis to be investigated. To search for possible coactivators for the AF-2 activator functions located in steroid receptor HBDs, we have used the yeast two-hybrid system (18) to identify proteins that interact with the HBD of the glucocorticoid receptor (GR). We report here the partial sequence of a novel protein, glucocorticoid receptor-interacting protein 1 (GRIP1), that interacts with steroid receptor HBDs in a hormone-dependent manner. We also provide evidence that GRIP1 can interact functionally with the transcription machinery *in vivo* and serve as a coactivator for the transcriptional activation function of steroid receptor HBDs.

MATERIALS AND METHODS

Construction of Recombinant Vectors. The following yeast two-hybrid system vectors were obtained from Clontech: pGBT9, for expressing GAL4–DBD fusion proteins and pGAD424 and pGAD10, for expressing GAL4–AD fusion proteins. Yeast expression vectors for DBD_{GAL4}–$HBD_{steroid\ receptor}$ fusion proteins, named pGBT9.HBD_{GR}, pGBT9.HBD_{ER}, and pGBT9.HBD_{AR}, were made by inserting PCR-amplified cDNA fragments coding for mouse $GR_{513-783}$, human estrogen receptor ($ER_{274-595}$), and human androgen receptor ($AR_{644-919}$) into *Sma*I/*Sal*I, *Eco*RI/*Sal*I, and *Bam*HI/*Pst*I sites, respectively, in pGBT9. pGAD10.GRIP1 was originally isolated from a Clontech Matchmaker 17-day-old mouse embryo cDNA library by screening (see below). pGBT9.GRIP1 was made by subcloning the *Eco*RI fragment (coding for GRIP1) of pGAD10.GRIP1 into the *Eco*RI site of pGBT9. Yeast expression vector pGRIP1 was made by removing the *Kpn*I–*Eco*RI fragment (coding for GAL4 AD) of pGAD424, replacing it with CGCCGCCCTCGAGG, and then subcloning the *Eco*RI fragment coding for GRIP1 into the *Eco*RI site of this new vector. The mammalian expression vector for GRIP1, pCMV.HA/GRIP1, was made in several steps. First, a double-stranded oligonucleotide with coding strand AATTAGATCTGGATCCGCCGCCATGGGCTACCCATACGATGTTCTTGACTATGCGGAATTC, which encodes an *Eco*RI cohesive end, *Bam*HI site, translation start signal (underlined), the hemagglutinin epitope tag YPYDVPDYA (19) (underlined), a new *Eco*RI site, and a *Bam*HI cohesive end (in the complementary strand), was inserted into the *Eco*RI/*Bam*HI sites in pBlueScript (Stratagene); this destroyed the original *Eco*RI and *Bam*HI sites. Second, the *Eco*RI fragment encoding GRIP1 was inserted into the new unique *Eco*RI site. Third, the *Bam*HI–*Xba*I fragment from this modified pBlueScript plasmid was inserted into *Bgl*II/*Nhe*I sites of pCMV, which was derived from pCMV.Neo (20). Mammalian expression vectors for the GR DBD and a DBD_{GR}–GRIP1 fusion protein, named pCMV.DBD_{GR} and pCMV.DBD_{GR}/GRIP1, respectively, were made as follows: PCR was used to generate a DNA fragment with *Xho*I site, *Bam*HI site, translation start signal, coding region for mouse GR DBD and nuclear localization signal (amino acids 393–512), *Eco*RI site, stop codons in all three reading frames, and *Xba*I site. This fragment was inserted into *Xho*I/*Xba*I sites in pBlueScript, generating pBS.DBD_{GR}. The *Eco*RI fragment coding for GRIP1 was subcloned into the *Eco*RI site, generating pBS.DBD_{GR}/GRIP1. The *Bam*HI–*Xba*I fragments from pBS.DBD_{GR} and pBS.DBD_{GR}/GRIP1 were subcloned into *Bgl*II/*Nhe*I sites of pCMV to create pCMV.DBD_{GR} and pCMV.DBD_{GR}/GRIP1. pGEX1.GRIP1, a bacterial expression vector coding for a GST–GRIP1 fusion protein, was made by inserting a PCR-amplified fragment coding for $GRIP1_{415-812}$ into the *Eco*RI site in pGEX1 (Amrad, Kew, Victoria, Australia).

Use of Two-Hybrid System To Isolate GRIP1 cDNA Clone and Study GRIP1 Function in Yeast. The Matchmaker Two-Hybrid System kit (Clontech protocol PT1265-1) was used to screen a Matchmaker 17-day-old mouse embryo cDNA library (Clontech protocol PT1020-1) according to the manufacturer's protocols; the library was in pGAD10. Screening was performed in the presence of 10 μM deoxycorticosterone (DOC), using pGBT9.HBD_{GR} as bait. The 2.5-kb GRIP1 cDNA insert was sequenced with a Sequenase version 2.0 DNA Sequencing kit (United States Biochemical). All data from the yeast system were obtained using yeast strain SFY526 (21). Plasmids pVA3 and pTD1 (Clontech), encoding DBD_{GAL4}–p53 and AD_{GAL4}–T-antigen fusion proteins, respectively, were used as negative controls where indicated. Units of β-galactosidase (βgal) activity are defined by the formula, $1000 \times OD_{420}/(t \times V \times OD_{600})$, where OD_{420} is from the *o*-nitrophenyl β-D-galactosidase/hydrolysis assay; *t*, time of incubation in min; V, volume of yeast culture, from which the assayed extract sample was prepared; and OD_{600}, absorbance at 600 nm of 1 ml of the yeast culture.

Interaction of GRIP1 and Steroid Receptors *in Vitro*. Glutathione *S*-transferase (GST) and GST–GRIP1 fusion proteins were produced in *Escherichia coli* carrying pGEX1 or pGEX1.GRIP1, respectively, bound to glutathione-Sepharose beads, and analyzed for binding with labeled proteins as described previously (22), except that the NETN buffer (22) used for binding and washing contained only 0.01% Nonidet P-40 and no powdered milk. The labeled proteins were synthesized by transcription and translation *in vitro* as described (23) except that [^{35}S]methionine was included in the translation reaction. The unpurified labeled proteins were incubated with glutathione-Sepharose beads, and after washing, the labeled proteins bound to the beads were eluted by boiling in SDS sample buffer (24) and analyzed by SDS/PAGE and autoradiography.

Mammalian Cell Transient Transfection Assays. Cells were transfected by the calcium phosphate method as described (25), except that 6-well culture dishes (34-mm diameter wells) were used. Expression vectors for reporter genes were pMMTV-CAT (26); pCMV-βgal, which was derived from pCMV.Neo (20); and pArg-maxigene, a derivative of a $tRNA^{arg}$ gene containing an additional 12 bp inserted between the internal promoter regions (27). Chloramphenicol acetyltransferase (CAT) assays were performed by a phase-separation method (28) and βgal assays were performed as described (29). Ribonuclease protection assays to detect the modified $tRNA^{arg}$ were performed as described (30) except that 0.5 μg of total cell RNA was analyzed in each assay.

RESULTS

Isolation of a Partial cDNA for GRIP1, A Steroid Receptor-Interacting Protein. The yeast two-hybrid system was used to identify proteins encoded in a 17-day-old mouse embryo cDNA library that interact with the GR HBD. The coding sequences for the hinge region and complete HBD of the mouse GR (amino acids 513–783) (26) were cloned into a yeast expression vector to create a gene encoding a DBD_{GAL4}–HBD_{GR} fusion protein. This vector was transformed stably into yeast along with a second vector that expressed fusion proteins encoded by the GAL4 AD cDNA and the mouse embryo cDNA clones. Interaction of the two fusion proteins may result in the reconstitution of a functional GAL4 protein, which can subsequently activate a βgal reporter gene containing an enhancer element recognized by the GAL4 DBD. About 4×10^6 yeast transformants were screened in the presence of 10 μM DOC, a strong glucocorticoid agonist in yeast (31). Among 15 positive cDNA clones, three clones with identical restriction maps were designated as GRIP1. The GRIP1 protein fragment interacted specifically with the GR HBD in the yeast two-hybrid system in the presence but not in the absence of DOC (Fig. 1), indicating that the interaction is hormone regulated. When the GR HBD was replaced with the HBD of the human ER (amino acids 274–595) (32) or AR (amino acids 644–919) (33), a specific hormone-dependent interaction with GRIP1 was also observed in the two-hybrid system (Fig. 1). In two-hybrid system controls lacking hormone, or where the steroid receptor HBD or GRIP1 were replaced by other proteins (Fig. 1), activity of the βgal reporter gene was undetectable (<0.2 unit).

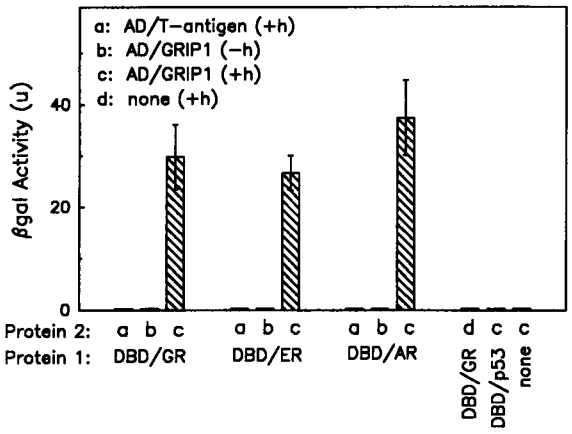

FIG. 1. Interaction of GRIP1 with steroid receptor HBDs in a yeast two-hybrid system assay. The indicated fusion proteins were stably expressed in yeast grown in solution culture in the absence or presence of the appropriate steroid hormone (10 μM DOC for GR, 100 nM estradiol for ER, and 100 nM dihydrotestosterone for AR). Interaction of the two proteins reconstituted the two functional GAL4 domains and resulted in activation of an endogenous βgal reporter gene with a GAL4 enhancer element. βgal activity of cell extracts from liquid yeast cultures is shown. Each point is the average from three independent yeast transformants. DBD, GAL4 DBD; AD, GAL4 AD; h, hormone.

The entire 2.5-kb cDNA insert of GRIP1 was sequenced and found to encode an 812-aa long fragment; the open reading frame spanned the entire length of the insert (Fig. 2). Comparison of the GRIP1 DNA and protein sequences with GenBank by using National Institutes of Health (Bethesda) BLAST programs (34) showed no significant homology between GRIP1 and any reported DNA and protein sequences. Northern blot analyses using the GRIP1 insert as a probe identified RNA species of ≈9-kb and 7-kb in mouse 12- to 17-day-old embryos and in all adult mouse tissues tested, including brain, heart, lung, muscle, skin, and spleen (data not shown).

Interaction of GRIP1 and Steroid Receptor HBDs *in Vitro*. To further characterize the GRIP1-steroid receptor binding observed in the yeast two-hybrid system, we studied the interaction of these proteins *in vitro*. Amino acid residues 415–812 of the originally identified GRIP1 fragment were expressed in *E. coli* as a fusion protein with GST. We had previously determined in the yeast two-hybrid system that this GRIP1 fragment binds steroid receptor HBDs (data not shown). The GST–GRIP1$_{415-812}$ fusion protein was adsorbed from the bacterial extract onto glutathione-Sepharose beads and incubated with ^{35}S-labeled GR, ER, or AR protein fragments, consisting of the complete DBD and HBD, which had been synthesized *in vitro* in the presence or absence of a suitable agonist (DOC for GR, estradiol for ER, and dihydrotestosterone for AR). Bound material was eluted and analyzed by SDS/PAGE and autoradiography. The GR, ER, and AR fragments did not bind to GST protein alone, but bound strongly to GST/GRIP1$_{415-812}$ in the presence of appropriate hormones and weakly in the absence of hormones (Fig. 3). Because GRIP1–HBD interaction was strictly hormone dependent in the yeast two-hybrid system, it is likely that the weak but specific binding observed *in vitro* in the absence of hormone is due to partial dissociation of the steroid receptor fragments from hsp90 *in vitro*. A similar observation was made by Cavaillès *et al.* (35) for the interaction between RIP140 and ER HBD.

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EFAAGRHHHE VLRQGLAFSQ IYRFSLSDGT LVAAQTKSKL IRSQTTNEPQ
LVISLHMLHR EQNVCVMNPD LTGQAMGKPL NPISSSSPAH QALCSGNPGQ (100)
DMTLGSNINF PMNGPKEQMG MPMGRFGGSG GMNHVSGMQA TTPQGSNYAL
KMNSPSQSSP GMNPGQASSV LSPRQRMSPG VAGSPRIPPS QFSPAGSLHS (200)
PVGVCSSTGN SHSYTNSSLN ALQALSEGHG VSLGSSLASP DLKMGNLQNS
PVNMNPPPLS KMGSLDSKDC FGLYGEPSEG TTGQAEASCH PEEQKGPNDS (300)
SMPQAASGDR AEGHSRLHDS KGQTKLLQLL TTKSDQMEPS PLPSSLSDTN
KDSTGSLPGP GSTHGTSLKE KHKILHRLLQ DSSSPVDLAK LTAEATGKEL (400)
SQESSSTAPG SEVTVKQEPA SPKKKENALL RYLLDKDDTK DIGLPEITPK
LERLDSKTDP ASNTKLIAMK TVKEEVSFEP SDQPGSELDN LEEILDDLQN (500)
SQLPQLFPDT RPGAPTGSVD KQAIINDLMQ LTADSSPVPP AGAQKAALRM
SQSTFNNPRP GQLGRLLPNQ NLPLDITLQS PTGAGPFPPI RNSSPYSVIP (600)
QPGMMGNQGM LGSQGNLGNN STGMIGSSTS RPSMPSGEWA PQSPAVRVTC
AATTGAMNRP VQGGMIRNPT ASIPMRANSQ PGQRQMLQSQ VMNIGPSELE (700)
MNMGGPQYNQ QQAPPNQTAP WPESILPIDQ ASFASQNRQP FGSSPDDLLC
PHPAAESPSD EGALLDQLYL ALRNFDGLEE IDRALGIPEL VSQSQAVDAE (800)
QFSSLEVDGR EF (812)
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FIG. 2. Partial amino acid sequence of GRIP1 predicted from the cDNA sequence.

Overexpression of GRIP1 Fragment in Mammalian Cells Inhibits Expression from RNA Polymerase II Promoters but not from RNA Polymerase III Promoters. If GRIP1 can interact with steroid receptors in mammalian cells, then overexpression of the GRIP1 fragment may perturb expression of steroid hormone-regulated genes. The entire GRIP1 coding fragment was inserted after an artificial translation start signal and under the control of a cytomegalovirus (CMV) promoter; this expression vector was transfected into mouse L cells along with three plasmids containing reporter genes: MMTV-CAT, which could be activated by the endogenous L cell GR in the presence of hormone; CMV-βgal, which should be expressed constitutively; and pArg-maxigene, a modified tRNA gene that should be expressed constitutively by RNA polymerase III. Expression of both MMTV-CAT and CMV-βgal was severely inhibited by the overexpressed GRIP1 fragment in a dose-dependent manner; however, in the same transfected cultures, expression from the RNA polymerase III promoter was not affected by the GRIP1 fragment (Fig. 4). Because it was unaffected, expression of the pArg-maxigene also served as an internal control for transfection efficiency. These results suggest that GRIP1 fragment overexpression generally interfered with transcription by RNA polymerase II, but not by RNA polymerase III. Such an effect could be due to squelching by excess GRIP1 fragment, i.e., binding of GRIP1 to an essential RNA polymerase II-specific transcription factor. Overexpression of a wide variety of transcription factors, including steroid receptors, has been shown to cause this type of squelching (36).

GRIP1 Has a Transcriptional Activation Domain that Functions in Both Yeast and Mammalian Cells. The suggestion that GRIP1 may interact with essential transcription factors raised the question of whether GRIP1 may contain an AD. Such an activity is generally identified by fusing the protein in question to the DBD of another protein and testing

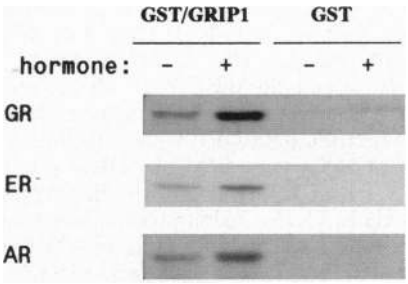

FIG. 3. Interaction of GRIP1 with steroid receptor HBDs *in vitro*. ^{35}S-labeled steroid receptor fragments, including the complete DBD and HBD, were synthesized *in vitro* in the presence or absence of an appropriate steroid agonist (see Fig. 1) and then incubated with Sepharose beads containing bound GST/GRIP1$_{415-812}$ or GST protein. The beads were washed and bound protein was eluted and analyzed by SDS/PAGE and autoradiography.

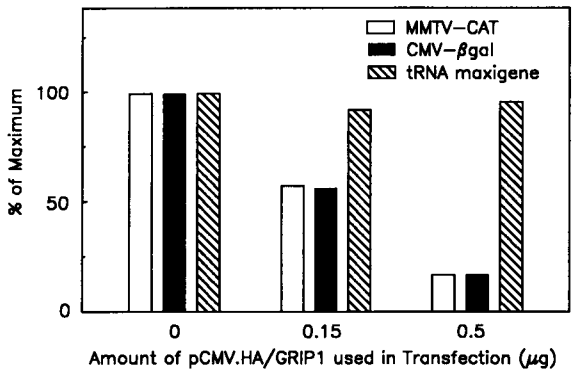

FIG. 4. Effect of GRIP1 overexpression on expression of cotransfected reporter genes. Mouse L cells were simultaneously cotransfected with the three indicated reporter genes and variable amounts of pCMV.HA/GRIP1. Cells were grown with 1 μM dexamethasone for the last 20 h before harvest and were harvested 48 h after transfection. Extracts from one set of transfected cells were assayed for CAT and βgal activity, while RNA preparations from a parallel set were subjected to RNase protection assays for the modified tRNA encoded by pArg-maxigene. Each bar represents the average of two independent transfections. CAT activity was completely dependent on glucocorticoid addition, while activity of the other two reporter genes was glucocorticoid independent.

whether the fusion protein can activate a reporter gene containing an enhancer site recognized by the DBD. When a DBD_{GAL4}–GRIP1 fusion protein was expressed in yeast, the βgal reporter gene controlled by a GAL4 enhancer site was strongly activated, whereas neither the GAL4 DBD nor GRIP1 alone was able to activate βgal expression (Fig. 5*A*). To test whether GRIP1 has transactivation activity in mammalian cells, we transiently expressed a DBD_{GR}–GRIP1 fusion protein in CV-1 cells. A cotransfected MMTV-CAT reporter gene was strongly activated by DBD_{GR}/GRIP1, but not by either GRIP1 or the GR DBD alone (Fig. 5*B*). Thus, GRIP1 fragment contains a transcriptional AD.

GRIP1 Can Function as a Coactivator for Steroid Receptor HBDs in Yeast. The observations that GRIP1 interacted with steroid receptor HBDs in a hormone-dependent manner and exhibited a transcriptional activation function when fused to a protein with a DBD suggest that GRIP1 may be able to serve as a coactivator for the transcriptional activation subdomains in steroid receptor HBDs. To test this possibility, the GRIP1 fragment was coexpressed in yeast with DBD_{GAL4}–$HBD_{steroid\ receptor}$ fusion proteins in the presence and absence of appropriate steroid ligands. While the GRIP1 fragment and the DBD_{GAL4}–HBD fusion proteins were inactive when expressed individually, their coexpression in yeast dramatically activated the βgal reporter gene in a hormone dependent manner (Fig. 6). Thus, in yeast GRIP1 can serve as a transcriptional coactivator for steroid receptor HBDs.

DISCUSSION

The mechanism by which transcriptional activator proteins, including steroid receptors, interact with the transcription machinery to activate the expression of adjacent genes is one of the most important unanswered questions in the field of gene regulation. DNA-bound transcriptional activators may exert their effects by making crucial protein–protein contacts with basal transcription factors, transcriptional coactivators, or chromatin components (1, 6). The GRIP1 protein fragment exhibited several characteristics of transcriptional coactivators. First, GRIP1 interacted with HBDs of three different steroid receptors in a hormone-regulated manner in yeast (Fig. 1) and *in vitro* (Fig. 3), indicating that GRIP1 recognizes a conserved hormone regulated feature of steroid receptor HBDs. It is interesting to note that the ER HBD shares only about 25% amino acid sequence identity with the other four steroid receptor HBDs (37). Second, when tethered to the enhancer element of a reporter gene by fusion to a suitable DBD, GRIP1 exhibited a strong transcriptional activation activity both in yeast and in mammalian cells (Fig. 5). This activity indicates that GRIP1 can interact functionally with the transcription machinery, a conclusion that is strengthened by the ability of overexpressed GRIP1 fragment to interfere specifically with RNA polymerase II-mediated transcription of transiently transfected reporter genes in mammalian cells (Fig. 4).

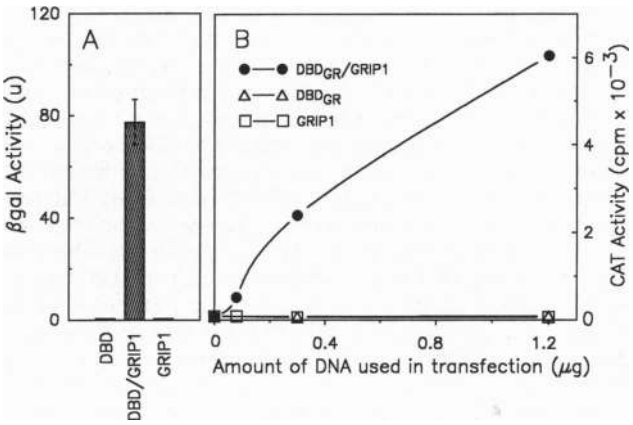

FIG. 5. Transcriptional activation by GRIP1 fragment fused with heterologous DNA binding domains. (*A*) Liquid yeast cultures stably expressing the indicated fusion or control protein and containing a βgal reporter gene with a GAL4 enhancer element were harvested, and extracts were assayed for βgal activity. DBD, GAL4 DBD. (*B*) CV-1 cells were transiently cotransfected with expression plasmids for the indicated fusion or control protein and for the reporter genes MMTV-CAT and CMV-βgal. After 48 h, cell extracts were made and assayed for CAT and βgal activity. Each point in *A* and *B* represents the average of two or three independent transfections, and each experiment has been reproduced independently with essentially identical results. βgal activity did not vary more than 2-fold among all transfected cultures.

Finally, a more specific indication of the possible function of GRIP1 was the ability of the GRIP1 fragment to serve as a strong transcriptional coactivator for the HBDs of steroid receptors in yeast (Fig. 6). While similar tests in mammalian cells with the full-size GRIP1 protein will be important to

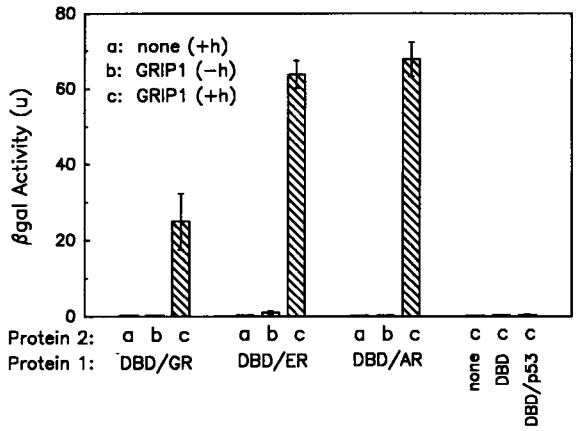

FIG. 6. GRIP1 can function as a coactivator for steroid receptor HBDs in yeast. The indicated proteins were stably expressed in yeast and their ability to activate the endogenous βgal reporter gene was determined. Yeast culture, hormone treatments, and βgal assays were performed as described in Fig. 1. DBD, GAL4 DBD; h, hormone. Each negative control (− hormone, − GRIP1, or − HBD) produced βgal activity of <0.2 unit, except that the activity for DBD/ER in column b was 1 unit.

determine its true physiological role, our efforts to conduct such tests have been complicated by the fact that steroid receptor HBDs exhibit transactivation activity in mammalian cells (3, 4). The strength of HBD transactivation activity in mammalian cells varies with cell and promoter context (38). However, in the yeast system, intact steroid receptors can strongly activate reporter genes with suitable enhancer elements (39), but the individual AF-1 (N-terminal) and AF-2 (HBD) activation domains of steroid receptors exhibit little or no activity under many conditions, when fused with a suitable DBD (Figs. 1 and 6) (40). Interestingly, the absence of HBD transactivation activity in yeast and the dramatic restoration of this activity by coexpression of the GRIP1 fragment suggest that yeast lack a coactivator that can mediate the transactivation activity of steroid receptor HBDs, even though many components of the transcription machinery of yeast and higher eukaryotes are highly conserved. By restoring this deficiency with GRIP1, we have established that yeast provide a convenient and powerful system for further investigations of steroid receptor coactivators.

Several other proteins that specifically interact with HBDs of steroid/thyroid hormone receptor superfamily members in a hormone regulated manner have been recently reported (35, 41–45). Aside from their ability to interact with nuclear receptors, no other activities have been observed for many of these proteins. The exceptions are RIP140 (35) and TIF1 (45), both of which exhibited some coactivator function for steroid receptors; expression of RIP140 in mammalian cells and TIF1 in yeast resulted in 2- to 3-fold enhancements of transcriptional activation by one or more nuclear receptors. In contrast, in our studies the undetectable transactivation activity of steroid receptor HBDs in yeast was dramatically enhanced at least 100-fold by coexpression of the GRIP1 fragment. Furthermore, the strong transcriptional activation function of GRIP1 when fused with a DBD and its ability to inhibit transcription from multiple RNA polymerase II-dependent promoters suggest an interaction between GRIP1 and the transcription machinery. This also contrasts with studies on RIP140, TIF1, and the other previously reported candidate coactivators for steroid receptors, for which no evidence of interaction with the transcription machinery has been provided. The mechanism by which coactivators mediate transactivation is unknown, although it is generally assumed that the coactivator may act as a physical bridge between the DNA-bound transcriptional activator protein and the basal transcription complex. The coactivation, transcriptional activation, squelching, and hormone dependent HBD-binding activities of GRIP1 are consistent with this model. The combination of these traits makes GRIP1 a very strong candidate for a transcriptional coactivator of steroid receptors.

Note Added in Proof. After submission of this paper, Oñate *et al.* (46) reported a novel coactivator for steroid hormone receptors, SRC-1. We find that GRIP1 and SRC-1 are partially homologous, indicating that they are distinct but related proteins.

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