Synergistic Effects of Coactivators GRIP1 and β-Catenin on Gene Activation

CROSS-TALK BETWEEN ANDROGEN RECEPTOR AND Wnt SIGNALING PATHWAYS*

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The p160 coactivators, such as GRIP1, bind nuclear receptors and help to mediate transcriptional activation. β-Catenin binds to and serves as a coactivator for the nuclear receptor, androgen receptor (AR), and the Lymphoid Enhancer Factor/T Cell Factor family member, Lef1. Here we report that GRIP1 and β-catenin can bind strongly to each other through the AD2 domain of GRIP1. Furthermore, GRIP1 and β-catenin can synergistically enhance the activity of both AR and Lef1, and both coactivators are recruited specifically to AR-driven and Lef1-driven promoters. However, the mechanism of β-catenin-GRIP1 coactivator function and synergy is different with AR and Lef1. While β-catenin can bind directly to both AR and Lef1, GRIP1 can only bind directly to AR; the ability of GRIP1 to associate with and function as a coactivator for Lef1 is entirely dependent on the presence of β-catenin. Thus, whereas GRIP1 coactivator function involves direct binding to nuclear receptors and most other classes of DNA-binding transcriptional activator proteins, the coactivator function of GRIP1 with Lef1 follows a novel paradigm where GRIP1 is recruited indirectly to Lef1 through their mutual association with β-catenin. The β-catenin-GRIP1 interaction represents another potential point of cross-talk between the AR and Wnt signaling pathways.

The androgen receptor (AR)† belongs to the nuclear receptor family of DNA-binding transcriptional activator proteins (1). Binding of the natural steroid hormones testosterone or dihydrotestosterone (DHT) to AR causes the protein to translocate from cytoplasm to nucleus where it binds to the promoters of androgen target genes and recruits specific coactivators to activate transcription. Among the many coactivators that contribute to the transcriptional activation process (2–4), the steroid receptor coactivator/nuclear receptor coactivator (SRC/NcoA/p160) family, encoding proteins of ~160 kDa, play central roles by serving as scaffold proteins or primary coactivators that bind directly to the DNA-bound nuclear receptors and recruit secondary coactivators, including histone-modifying enzymes. The three members of the p160 family are SRC-1, GRIP1/TIF-2, and p/CIP/AIB1/ACTR/RAC3/TRAM1 (2–4). All have a highly conserved N terminus, three nuclear receptor interaction motifs (LXXLL, where L is leucine and X is any amino acid) in the middle of the polypeptide chain, and two activation domains (AD1 and AD2) near the C terminus. AD1 and AD2 transmit the activating signal to the transcription machinery by binding to secondary coactivators: AD1 binds the histone acetyltransferases CBP and p300, while AD2 recruits the arginine-specific histone methyltransferase CARM1 (5). The multifunctional protein CBP/p300 serves as a coactivator for numerous transcriptional activators, including nuclear receptors such as hormone-activated AR (6) and the lymphoid enhancer factor/T cell factor (LEF/TCF) family proteins (7) through its intrinsic histone acetyltransferase activity and also perhaps through its ability to interact with basal transcription factors associated with RNA polymerase II (8–10). CARM1 contributes to transcriptional activation by methylation of specific arginine residues of histone H3 (11) and possibly by methylation of or protein-protein interactions with other components of the transcription machinery (12, 13). The AR signaling pathway is critical in the progression of prostate cancers, even in the androgen-independent stage in which androgen deprivation is no longer effective in blocking the transcription of AR target genes such as PSA (1, 14). Androgen independence can be caused by amplification of the AR gene, the emergence of AR mutants with a broadened spectrum of ligand agonists, or overexpression of p160 coactivators (1, 14, 15). In addition, altered regulation of other signaling pathways can contribute to androgen-independent proliferation of prostate cancer; for example, mutant β-catenin was found in one study in 5% of primary prostate cancers and thus may also contribute to unregulated cell growth in prostate cancer (16). β-Catenin is a key component of the Wnt signaling pathway, in which it serves as a coactivator for LEF/TCF transcriptional activator proteins (17), but β-catenin also binds to and serves as a coactivator for AR (18–22). β-Catenin is an armadillo motif-containing protein that is involved in regulation of cell-cell adhesion and transcriptional activation (17). β-Catenin protein levels are usually maintained at a low level through active degradation mediated by the glycogen synthase kinase (GSK) complex that contains adenomatous polyposis coli (APC) and axin. Upon the activation of the Wnt signaling pathway, GSK kinase activity is inhibited as the GSK complex
is dissociated; this allows β-catenin to bypass the destruction pathway. Increased accumulation allows some β-catenin to translocate to the nucleus where it binds to LEF/TCF proteins and enhances transcriptional activation of Wnt target genes, including c-Myc, cyclin D1, and ITF-2. Similarly, nuclear β-catenin also interacts with hormone activated AR (18) and retinoic acid receptor (23) and activates the transcription of their specific target genes. Binding of β-catenin to LEF/TCF causes displacement of coressor complexes containing histone deacetylases (24), and recruitment of CBP and p300, their specific target genes. Binding of β-catenin to LEF/TCF proteins for different enhancer element-binding transcriptional activators is important questions that remain to be addressed.

β-Catenin binds in an agonist-dependent manner to the AF-2 activation function within the hormone binding domain of AR; the AF-2 region of AR also contains another distinct binding site for GRIP1 (19). VP16-fused β-catenin was shown to modulate the ability of GRIP1 to enhance transcriptional activation by GAL4-DBD fused to the AR hormone binding domain (19). However, it remains unknown whether the intrinsic activation function of β-catenin cooperates with p160 coactivators in AR-mediated transcriptional activation. Here we demonstrate that the p160 coactivator GRIP1 binds strongly to β-catenin in vitro and in vivo, independent of AR, through the GRIP1 AD2 domain. We also show that the β-catenin-GRIP1 binding is important for their synergistic coactivator function with AR and Lef1. However, the manner in which these two coactivators are recruited to the AR and Lef1 transcription initiation complexes is different, since GRIP1 does not interact directly with Lef1. The β-catenin-GRIP1 interaction represents another potential point of cross-talk between the AR and Wnt signaling pathways.

MATERIALS AND METHODS

Cell Culture—CV-1 and COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen), and LNCaP cells were cultured in RPM11640. Both media were supplemented with penicillin and streptomycin and 10% fetal bovine serum.

Plasmids—Plasmids pSG5.HA-GRIP1, pSG5.HA-GRIP1(1-121), pVP16-GRIP1, pVP16-GRIP1(1122-1462), pGEX-4T1-GRIP1(5-765), pGEX1-GRIP1(730-1211), pGEX-4T1-GRIP1(1122-1462), and pSV40 AR were previously described (22, 23). The PCR-amplified Lef1 coding regions from pMVLNLeF1 were digested with EcoRI and BamHI and inserted into EcoRI and BglII sites of pSG5.HA and EcoRI and BamHI sites of pM; for bacterial expression, the EcoRI-XhoI β-catenin cDNA fragment was inserted into the homologous sites of pGEX-4T1.

Transfection—For co-immunoprecipitation assay, COS-7 cells were transiently transfected in 100-mm dishes as described previously (34). For reporter co-immunoprecipitation assays, COS-7 cells were transfected with 1 μg of each protein expression vector and 2 μg of reporter plasmids. After 48 h, a soluble chromatin fraction was prepared according to chromatin immunoprecipitation assay protocol (see below). For reporter gene assays, CV-1 cells were transiently transfected in 12-well plates as previously described (35).

Results

GRIP1 Binds β-Catenin, and Both Act Synergistically as Coactivators for AR—β-Catenin and GRIP1 bind to distinct sites in the AR AF-2 activation domain in a ligand-dependent manner (19). Moreover, each protein can enhance transcriptional activation by AR (18, 30). We therefore tested whether GRIP1 and β-catenin can act synergistically as coactivators for AR in transient transfection assays with a luciferase reporter plasmid controlled by the MMTV promoter. β-Catenin-enhanced DHT-activated AR activity 2.5-fold, and GRIP1 increased activity 4.4-fold in CV-1 cells (Fig. 1). When these two coactivators were co-expressed, a synergistic 25-fold activation was observed. The synergy suggests that the functions of GRIP1 and β-catenin are not redundant, and thus that they have different downstream targets or contribute to transcriptional activation in different ways.
Since GRIP1 and β-catenin were shown to bind to distinct sites in the AR AF-2 domain, it was previously assumed that the association between GRIP1 and β-catenin was through the androgen receptor (19). However, we tested whether GRIP1 and β-catenin might bind to each other even in the absence of AR. To test for binding in *in vitro* GRIP1 was translated in *vitro* in the presence of [35S]methionine (Fig. 2A, lane 1) and incubated with agarose beads containing bound GST or GST-β-catenin. GRIP1 bound strongly to GST-β-catenin (lane 3), but only background binding was observed with GST (lane 2). Since no AR or DHT was present, these results suggest a direct interaction between GRIP1 and β-catenin. β-Catenin also bound to the two other members of the p160 family, SRC-1 and ACTR, in similar GST pull-down assays (data not shown), indicating that binding to β-catenin is a conserved function of p160 coactivators.

We next tested for binding in *vitro* by co-expressing GRIP1 and β-catenin proteins in COS-7 cells, monkey kidney cells with no endogenous AR. Both HA-tagged GRIP1 and HA-tagged β-catenin were expressed well in COS-7 cells (Fig. 2B, top panel), and anti-GRIP1 antibodies effectively precipitated HA-GRIP1 (bottom panel). In the absence of exogenously expressed HA-GRIP1, anti-GRIP1 antibody co-immunoprecipitated a low level of β-catenin (middle panel, lane 2), which might be mediated by endogenous GRIP1. No β-catenin was detected in the immunoprecipitates when the β-catenin expression vector was omitted from the transfection (middle panel, lane 1). However, a strong co-immunoprecipitated β-catenin band was observed when both proteins were overexpressed (middle panel, lane 3), which indicates a β-catenin-GRIP1 complex that is independent of AR and DHT.

Binding of endogenous GRIP1 and β-catenin was examined in LNCaP prostate cancer cells, which also express endogenous AR. While no GRIP1 or β-catenin was immunoprecipitated by normal rabbit IgG (Fig. 2C, lane 2), anti-GRIP1 antibody co-immunoprecipitated β-catenin with GRIP1 from LNCaP cells that were grown in charcoal-stripped serum without DHT (Fig. 2C, lane 3). When similar experiments were performed using lysates from LNCaP cells treated with DHT, no enhancement of the β-catenin-GRIP1 interaction was observed (data not shown). Taken together, these results indicate that there is a physiological, AR-independent interaction between GRIP1 and β-catenin.

The AD2 Domain of GRIP1 Is Required For Its Interaction with β-Catenin and For Their Synergistic Activation of AR—Although GRIP1 and β-catenin can each bind directly to AR, we asked whether their synergistic effect on AR function requires the binding between GRIP1 and β-catenin. We first defined the region of GRIP1 that binds β-catenin, by testing the ability of...
**Fig. 3.** The AD2 domain of GRIP1 is required for interaction with \(\beta\)-catenin and for their synergistic activation of AR. **A**, binding *in vitro*. \(\beta\)-catenin synthesized *in vitro* was incubated with beads containing the indicated GST fusion proteins in a GST-pull-down assay. Bound proteins are shown in lanes 2–5 and 10% of the input, in lane 1. **B**, binding *in vivo*. For mammalian two-hybrid assays CV-1 cells were transfected with 200 ng of GK1-LUC reporter plasmid; 200 ng of plasmid encoding Gal4 DBD or Gal4 DBD fused to \(\beta\)-catenin; 200 ng of plasmid encoding VP16 AD or VP16 AD fused to GRIP1 or GRIP1 AD2 (amino acids 1122–1462). The luciferase activity results shown are representative of three independent experiments. **C**, synergistic versus additive coactivator function. CV-1 cells were transfected with 200 ng of MMTV-LUC reporter plasmid, 100 ng pSV40-AR, 400 ng of pSG5.HA-\(\beta\)-catenin, 200 ng of pSG5.HA-GRIP1 or 200 ng of pSG5.HA-GRIP1 AD2 as indicated. Cells were grown in medium containing 100 nM DHT. The luciferase activity results shown are representative of four independent experiments. Numbers above the bars represent fold enhancement compared with the activity of AR alone.

In *in vitro*-translated \(\beta\)-catenin to bind to each of three different GRIP1 fragments fused to GST. Concentrations of these GST fusion proteins were checked by Coomassie Blue staining (data not shown), and similar amounts of each protein bound to beads were mixed with \(\text{35S}^\text{S}\)-labeled \(\beta\)-catenin. While GST, GST-GRIP1(5–765), and GST-GRIP1(730–1121) did not bind to \(\beta\)-catenin (Fig. 3A, lanes 2–4), a strong interaction between the C-terminal GRIP1 AD2 domain (amino acids 1122–1462) and \(\beta\)-catenin was observed (lane 5).

To test whether the \(\beta\)-catenin-GRIP1AD2 interaction also occurs *in vivo*, a mammalian two-hybrid assay was used. Gal4DBD-\(\beta\)-catenin activated a transiently transfected re-
porter plasmid containing Gal4 response elements, confirming the transcriptional activation function of β-catenin (Fig. 3B, lanes 2 and 3). This activity was enhanced by co-expression of VP16 fused to either full-length GRIP1 or GRIP1-(1122–1462) (AD2) (lanes 6 and 7), but not by VP16 fused to GRIP1 AD1 (data not shown), suggesting that the interaction between GRIP1 and β-catenin is specifically through the AD2 region in GRIP1. Based on these results, we then tested whether the GRIP1 AD2 region is important for synergistic coactivator function of GRIP1 and β-catenin in AR-mediated transcriptional activation. GRIP1 enhanced AR activity by 5-fold, β-catenin enhanced AR activity by 3-fold, and together they caused a synergistic 15-fold enhancement (Fig. 3C, lanes 3–6). GRIP1ΔAD2 (lacking AD2) alone enhanced AR activity 3-fold, but the effect of GRIP1ΔAD2 and β-catenin together was 7-fold, which is additive rather than synergistic (lanes 7 and 8). Thus, while GRIP1ΔAD2 was still able to bind AR (30) and enhance AR function, its inability to function synergistically with β-catenin as a coactivator correlated with its inability to bind β-catenin. These data suggest that the binding of β-catenin to GRIP1 through the GRIP1 AD2 domain is required for the synergistic coactivator function between GRIP1 and β-catenin.

β-Catenin Is Recruited by AR to Promoters of Transiently Transfected MMTV-LUC Reporter Gene and Endogenous PSA Gene in a Hormone-dependent Manner—To test whether the coactivator function of β-catenin involves recruitment of β-catenin to the promoter by AR, we employed a reporter co-immunoprecipitation (reporter co-IP) assay. AR and β-catenin were co-expressed in COS-7 cells in the presence of MMTV-LUC reporter gene. Chromatin immunoprecipitation assays were then performed with untreated or DHT-treated cells. Primers flanking the hormone response elements (AR binding sites) in the MMTV promoter were used in PCR reactions to detect this promoter in the chromatin and reporter gene fragments immunoprecipitated by antibodies against AR or β-catenin. Both AR (used as a positive control) and β-catenin were found to be associated with the transiently transfected MMTV promoter in a DHT-dependent manner (Fig. 4A). In contrast, equal, low background levels of PCR products were detected in normal IgG immunoprecipitates from chromatin of untreated and DHT-treated cells. PCR analysis of input chromatin samples taken before the immunoprecipitation confirmed that equal amounts of chromatin from DHT-treated and untreated cells were used for immunoprecipitations. Since GRIP1 interacts with AR and β-catenin, we next tested whether co-expression of exogenous GRIP1 with β-catenin and AR would enhance the recruitment of β-catenin to the MMTV promoter (Fig. 4B). While AR, GRIP1, and β-catenin were all recruited to the transiently transfected MMTV promoter in response to DHT, the co-expression of GRIP1 with AR and β-catenin did not obviously alter the recruitment of β-catenin to the promoter. This finding is consistent with the previous report that GRIP1 and β-catenin can bind to different sites of the AR AF2 region simultaneously (19).

Chromatin immunoprecipitation (ChIP) assays were also performed with LNCaP cells to examine DHT-induced assembly of the transcription complex on the promoter of the endogenous PSA gene, which contains three enhancer elements for AR binding. Antisera against AR, GRIP1, and β-catenin all precipitated the PSA promoter preferentially from DHT-treated versus untreated LNCaP cell chromatin (Fig. 4C). Together, the ChIP, reporter Co-IP, and β-catenin-GRIP1 binding studies indicate that a ternary complex of hormone-activated AR, GRIP1, and β-catenin is bound to AR response elements, where GRIP1 and β-catenin bind to each other and to AR and synergistically activate AR function.

GRIp1 Enhances β-Catenin-dependent Lef1-mediated Transcriptional Activation as a Secondary Coactivator—β-Catenin was originally identified as a coactivator for the LEF/TCF family of transcriptional activators. Since GRIP1 can bind to and synergistically cooperate with β-catenin to enhance AR-mediated transcriptional activation, we tested whether GRIP1 could also cooperate with β-catenin to enhance the function of Lef1. In transient transfection assays in CV-1 cells reporter gene pGL3OT, containing enhancer elements that bind LEF/TCF proteins, was modestly activated by overexpression of Lef1 (Fig. 5A, lanes 1 and 2) and further activated 7-fold by co-expression of β-catenin (lane 5). However, increasing amounts of GRIP1 expression only weakly (up to 2-fold) enhanced Lef1-mediated luciferase expression (lanes 3 and 4). In contrast, overexpression of GRIP1 and β-catenin together dramatically and synergistically increased reporter gene expres-
Substitution of Lef1ΔN67, an N-terminal deletion mutant that retains DNA binding activity but fails to interact with β-catenin (24), for wild-type Lef1 almost completely eliminated the coactivator effects of β-catenin and GRIP1 (lane 8), indicating that the binding of β-catenin to Lef1 is required for the coactivator effects of both β-catenin and GRIP1. Wild-type Lef1 and Lef1ΔN67 were expressed at similar levels (data not shown). In contrast to the pGL3OT reporter plasmid, control reporter plasmid pGL3OF, containing mutant LEF/TCF binding sites, was not activated by either β-catenin alone or β-catenin plus GRIP1 (Fig. 5A, open bars). Thus, the ability of β-catenin and GRIP1 to enhance reporter gene activity also depended on the recruitment of the enhancer binding protein Lef1 to the promoter.

GRIP1 and the other p160 coactivators are considered to be primary coactivators for nuclear receptors, because p160 coactivators bind directly to nuclear receptors and serve as a scaffold for recruitment of a variety of secondary coactivators that include histone modifying enzymes. Consistent with this model, p160 coactivators alone can enhance the activity of nuclear receptors in transient transfection assays, whereas the enhancing activity of the secondary coactivators depends on the co-expression of p160 coactivators (38). In contrast, effective activation of Lef1 by GRIP1 required the presence of exogenous β-catenin (Fig. 5A), suggesting that the recruitment of GRIP1 to the promoter by Lef1 is mediated by β-catenin. We therefore tested whether GRIP1 can bind to Lef1 in the absence of β-catenin in GST pull-down assays. β-Catenin synthesized in vitro bound strongly and specifically to GST-Lef1 (Fig. 5B, lower panel) but GRIP1 synthesized in vitro did not (upper panel).

Thus, GRIP1 did not bind to Lef1, and GRIP1 alone could not activate Lef1 effectively, suggesting that GRIP1 functions as a secondary coactivator for Lef1, recruited to Lef1 by β-catenin. To further evaluate this hypothesis, we tested whether β-cate-
nin can promote the formation of a ternary complex with GRIP1 and Lef1 in a modified mammalian two-hybrid assay. Interaction between Gal4DBD-Lef1 and VP16-GRIP1 fusion proteins was tested in the presence and absence of co-expressed β-catenin. In the absence of VP16-GRIP1, β-catenin modestly enhanced the ability of Gal4DBD-Lef1 to activate a reporter gene controlled by Gal4 response elements (Fig. 6A, lanes 4 and 5). In the absence of β-catenin, VP16-GRIP1 failed to enhance the activity of (i.e. failed to bind to) Gal4DBD-Lef1 (lanes 4 and 6). However, co-expression of β-catenin with the two fusion proteins elicited strong reporter gene activity (Fig. 6A, lane 7), indicating that β-catenin served as a bridge between GRIP1 and Lef1 to form a ternary complex.

As a further test of the hypothesis that GRIP1 acts as a β-catenin-dependent secondary coactivator for Lef1, we tested whether the AD2 domain of GRIP1, which is required for GRIP1 binding to β-catenin, is necessary for β-catenin-GRIP1 coactivator synergy with Lef1. Without β-catenin, neither full-length GRIP1 nor GRIP1ΔAD2 enhanced the ability of Lef1 to activate expression of the pGL3OT reporter gene (Fig. 6B, lanes 1–4), whereas β-catenin alone caused a 3-fold enhancement of Lef1 activity (lane 5). Full-length GRIP1 acted synergistically with β-catenin to enhance Lef1 activity, but GRIP1ΔAD2 failed to cooperate with β-catenin to enhance Lef1 function (Fig. 6B, lanes 5–7). Thus, the ability of GRIP1 to function as a coactivator for Lef1 depends completely on the presence of β-catenin and an intact GRIP1 AD2 domain, which is required for binding to β-catenin. In summary, while GRIP1 binds directly to and serves as a primary coactivator for nuclear receptors, GRIP1 acts as a secondary coactivator for Lef1-mediated transcriptional activation by using the primary coactivator β-catenin as the scaffold to connect GRIP1 to DNA-bound Lef1.

Recruitment of GRIP1 to Promoters Containing Lef1 Response Elements—To test whether GRIP1 and β-catenin are actually recruited to the Lef1-regulated promoter of the pGL3OT plasmid, reporter co-IP assays were performed on COS-7 cells transiently transfected with pGL3OT reporter plasmid and expression vectors for Lef1, β-catenin, and GRIP1. As an internal control, the pGL3-SV40 reporter plasmid, which has the same plasmid backbone as pGL3OT and contains an SV40 promoter but no Lef1 binding sites, was also included in the transfection. The chromatin immunoprecipitation procedure was performed with the transiently transfected cells, using normal rabbit IgG or antibodies against Lef1, β-catenin, or GRIP1. The DNA from the immunoprecipitates was analyzed by PCR using primers against sequences on the backbone of the pGL3-basic luciferase plasmid. These primers flank the Lef1
Fig. 7. GRIP1 is specifically recruited to Lef1 response elements in vivo. COS-7 cells were transiently transfected with 2 μg each of pGL3OT (containing three Lef1 response elements, LRE) and pGL3-SV40 (containing SV40 promoter but no Lef1 response elements); pSG5.HA-GRIP1 (1 μg); and pSG5.HA-β-catenin (1 μg). Reporter co-IP assays were performed using the indicated antibodies. The precipitated DNA was analyzed by PCR using primers that recognize identical backbone sequences in both reporter plasmids, as illustrated in the diagram. Results shown were performed with 2 μl of a 1:50 dilution of input and precipitated DNA and are representative of three independent experiments.

binding sites in pGL3OT plasmid and produce a 318-base pair PCR product; the same primers flank the SV40 promoter region in pGL3-SV40 plasmid and produce a 412-base pair PCR product (Fig. 7). Similar amounts of the two PCR products were generated when primers were used to amplify DNA from input chromatin (i.e. before immunoprecipitation), which demonstrates the coexistence of the two reporter genes in the transfected COS-7 cells (Fig. 7, lane 1). However, PCR analysis of DNA immunoprecipitated by antibodies against Lef1 (lane 2), β-catenin (lane 3), or GRIP1 (lane 4) indicated that the pGL3OT plasmid, which binds Lef1 was greatly enriched in the immunoprecipitates, compared with the pGL3-SV40 plasmid, which lacked Lef1 sites. Normal rabbit IgG precipitated equal low background levels of both plasmids (lane 5). Thus, Lef1, β-catenin, and GRIP1 were specifically recruited to the Lef1 response elements. This finding supports the model developed from the reporter gene activity assays that Lef1 recruits β-catenin and GRIP1 to the promoter, where they mediate the transcriptional activation process triggered by the binding of Lef1 to the promoter.

DISCUSSION

β-Catenin-GRIP1 Complex Synergistically Enhances AR Function—Increased levels of nuclear β-catenin, induced by the Wnt signaling pathway, stimulate LEF/TCF-mediated transcription of genes that control cellular growth, differentiation, and survival, such as c-Myc, cyclin D1, and TFF2 (17). Various mechanisms leading to aberrant stabilization of cytoplasmic β-catenin, and thus resulting in the uncontrolled nuclear localization of β-catenin and transactivation of Wnt target genes, have been found in colon cancer and hepatocellular carcinoma (HCC) (39, 40). Since β-catenin has recently been shown to bind to and enhance transcriptional activation by AR, we may expect to see reports of β-catenin involvement in prostate cancer. In fact, it was recently reported that about 5% of primary prostate cancers contain β-catenin mutations, which stabilize β-catenin and enhance its nuclear concentration (16). Until recently, the relationship between β-catenin and p160 coactivators, which both bind directly to AR and recruit secondary coactivators, has not been investigated. Since distinct bindings sites in the AR LBD for p160 coactivators and β-catenin were identified (19), it was hypothesized that the primary interaction between β-catenin and p160 coactivators was through AR (19). However, in this study we demonstrated a strong, AR-independent and hormone-independent interaction between GRIP1 and β-catenin through the AD2 domain of GRIP1 (Figs. 2 and 3); since this strong interaction occurred in vivo as well as in vitro, it is likely to involve direct contact between the two proteins. Since the synergistic coactivator function between GRIP1 and β-catenin required the GRIP1 AD2 domain, which binds β-catenin (Fig. 3), it is likely that this direct β-catenin-GRIP1 interaction is required for their synergistic function. Thus, in the ternary complex between AR, GRIP1, and β-catenin, the fact that each component can bind to the other two should produce a relatively stable complex, and this stability may contribute to the synergistic coactivator function, which we observed for GRIP1 and β-catenin.

GRIP1 Serves as a Secondary Coactivator for Lef1—GRIP1 enhanced the activity of Lef1 on the pGL3OT reporter gene by as much as 10-fold in the presence of β-catenin, but had almost no effect in the absence of β-catenin (Fig. 5A). The dependence of GRIP1 coactivator function on β-catenin, when the DNA-binding transcriptional activator is Lef1, is in stark contrast to the performance of GRIP1 with AR, where GRIP1 alone can substantially enhance AR function (Fig. 1) (30). The coactivator activity of GRIP1 with Lef1 was also eliminated by mutations in the Lef1 binding sites of the reporter gene, an N-terminal deletion of Lef1 that prevented binding to β-catenin, or deletion of the AD2 domain of GRIP1 which is responsible for binding β-catenin (Figs. 5A and 6B). These findings demonstrate that both the interaction between β-catenin and Lef1 and the interaction between β-catenin and GRIP1 are required for GRIP1 coactivator function with Lef1. In addition, GRIP1 was unable to bind Lef1 in vitro (Fig. 5B) and only bound Lef1 in vivo when β-catenin was present to form a bridge between GRIP1 and Lef1 (Fig. 6A). In combination, these findings demonstrate that GRIP1 functions as a secondary coactivator for Lef1.

p160 coactivators function as primary coactivators for (i.e. they bind directly to and enhance the activities of) several different classes of DNA binding transcriptional activators and use several different domains to interact with the different types of transcriptional activators. For example, the LXXLL motifs in the p160 central region bind AF-2 domains of many nuclear receptors (2); aryl hydrocarbon receptor binds to the AD1 region of p160 coactivators (41); and MEF-2C binds to the N-terminal region of p160 coactivators (42). Thus the relationship of GRIP1 with Lef1 is novel for two reasons: first, GRIP1 functioned as a secondary coactivator for Lef1; and second, the AD2 domain of GRIP1, which in some cases can be used to recruit secondary coactivators like CARM1 (and thus acts as a signal output domain), was responsible for coupling GRIP1 indirectly (through β-catenin) to the transcription factor Lef1 in this case (and thus acts as a signal input domain). Lef1 belongs to the LEF/TCF family, which is expressed in multiple tissues including breast and prostate (39). Therefore, involvement of p160 coactivators in the progression of breast cancer and prostate cancer, e.g. through overexpression (15, 43), might not be limited to their role in mediating the activation of genes by ER and AR, but may also extend to a role in mediating Lef1 target genes in cooperation with β-catenin in response to activation of the Wnt signaling pathway.

Cross-talk between AR and Wnt Signaling Pathways—The physiological significance of the β-catenin-GRIP1 complex was demonstrated by the association between endogenous GRIP1 and β-catenin proteins in LNCaP cells, which was detected in the absence of DHT, showing that it is independent of AR. The
same binding was observed in vitro and with overexpressed proteins in COS-7 cells, both in the absence of AR. Chromatin immunoprecipitation assays demonstrated that both GRIP1 and β-catenin were recruited in a hormone-dependent manner to the promoter of an endogenous AR target gene in its native chromosomal location, further confirming the physiological significance of the β-catenin-GRIP1 association. Although physical and functional interactions between β-catenin and AR have been previously demonstrated, our study provides the first evidence of β-catenin recruitment to the promoter of an endogenous AR responsive gene (Fig. 4C).

Both p160 coactivators and β-catenin bind directly to DNA-bound transcriptional activator proteins and function as scaffold proteins which use their activation domains to recruit secondary coactivators, including the histone acetyltransferases p300 and CBP and the histone methyltransferase secondary coactivators, including the histone acetyltransferases which use their activation domains to recruit bound transcriptional activator proteins and function as scaffolds. The direct interaction between AR DBD and Tcf4 may mediate mutual repression on each other, by competition for limited coactivator binding sites. These observations may prove to be particularly effective in recruiting the histone-modifying coactivators, and the enhanced recruitment of these secondary coactivators might in part account for the synergistic function of β-catenin and GRIP1 as coactivators. Furthermore, the relative cellular levels of p160 coactivators and β-catenin might determine the usage of either one or both of them in any given situation.

The fact that β-catenin is a coactivator for AR as well as LEF/TCF transcription factors provides a mechanism for cross-talk between the Wnt and AR signaling pathways. In addition, the use of common coactivators (e.g. β-catenin and CARMA1) by AR and Lef1 may explain why these two transcription factors exert mutual repression on each other, by competition for limiting coactivators (44, 45). Alternatively, a recently reported direct interaction between AR DBD and Tcf4 may mediate their mutual repression (45). Our demonstration that GRIP1 serves as a coactivator for Lef1 in addition to AR, and that GRIP1 and β-catenin associate with each other in vivo provides yet another point for cross-talk between the Wnt and AR signaling pathways.

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Synergistic Effects of Coactivators GRIP1 and β-Catenin on Gene Activation: CROSS-TALK BETWEEN ANDROGEN RECEPTOR AND Wnt SIGNALING PATHWAYS

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