Binding of Liganded Vitamin D Receptor to the Vitamin D Receptor Interacting Protein Coactivator Complex Induces Interaction with RNA Polymerase II Holoenzyme*

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Because the vitamin D receptor interacting protein (DRIP) coactivator complex shares components with the RNA polymerase II (Pol II) holoenzyme complex, we tested whether the two protein complexes associate in cellular extracts. On initial purification steps, the DRIP complex copurified with the Pol II holoenzyme. Pol II was found to bind to the vitamin D receptor in a ligand-dependent fashion when either nuclear extracts or partially purified preparations were used as sources of DRIP and Pol II holoenzyme. A subpopulation of holoenzyme complexes bound to the receptor because BRCA1, which associates with the Pol II holoenzyme, did not associate with the liganded receptor, and only in certain of the holoenzyme- and DRIP-containing fractions did Pol II bind to the liganded receptor. Immunoprecipitation experiments revealed that the DRIP complex was not pre-associated with the Pol II holoenzyme, but the interaction between these two complexes was induced only in the presence of receptor and ligand. These data support a model in which the activation of transcription by hormone-bound receptor requires binding to the DRIP coactivator, and this induced ternary complex can then bind to the Pol II holoenzyme to activate transcription.

Current models of transcriptional regulation in mammalian cells posit the RNA polymerase II (Pol II)1 holoenzyme mediating the control of mRNA synthesis from all protein-encoding genes (1, 2). Along with core Pol II, the Pol II holoenzyme contains basal transcription factors, such as TFIIIF, TFIIIE, and TFIIH, and multiple regulatory proteins, including CBP, p300, BRCA1, SRB factors, and mediator polypeptides (3).

Nuclear receptors, such as the vitamin D receptor, transduce hormonal signals by binding directly to DNA target sites in promoters, and when in the presence of sufficiently high concentrations of a specific ligand, the receptor directs the activation of gene transcription. Receiver binding to ligand induces conformational changes in the protein, creating a binding surface for cofactors that stimulate gene expression (reviewed in Ref. 4). The vitamin D receptor interacting proteins (DRIP) complex was found to bind to the nuclear receptor in a ligand-dependent fashion (5). This complex is classified as a coactivator because its binding to the receptor promotes the activation of transcription in reactions performed in vitro (6).

Several groups (6–13) have reported the identification from mammalian cells of coactivator complexes identical, or highly similar, to DRIP, variously named SMCC, TRAP, ARC, CRSP, NAT, and the mouse and human Mediators. The polypeptide compositions of these related coactivator complexes contain 9–20 polypeptides. Thus, for the sake of simplicity in the following text, the term “DRIP complex” will be used to connote all of these coactivator complexes because the experiments presented do not discriminate between the different complexes. Some of the polypeptides found in the DRIP complex are a subset of the SRB and mediator factors, such as Med7, previously thought to be exclusively present in the Pol II holoenzyme. Although the DRIP complex contains several mammalian SRB and mediator proteins, it lacks key components found in the Pol II holoenzyme, including the core Pol II enzyme, basal transcription factors, CBP, BRCA1, and several specific SRB factors such as Cdk8 (6, 7, 9). Thus, it is unclear whether the DRIP complex functions by binding to core Pol II to reconstitute a holoenzyme complex or whether it functions as a separate coactivator that interacts with the Pol II holoenzyme.

In the following experiments, we find that the DRIP complex is separable from the Pol II holoenzyme complex, but, strikingly, we found that the Pol II holoenzyme could be recruited to the vitamin D receptor dependent upon the presence of the ligand and the DRIP complex. In addition, data are presented demonstrating that only a subset of the Pol II holoenzyme complex associates with the receptor/ligand/DRIP, suggesting that the DRIPs do not merely reconstitute holoenzyme but also that they must function as a separable coactivator.

MATERIALS AND METHODS

Biochemical Purification of Mammalian Holoenzyme—HeLa cells were passaged in suspension culture using standard procedures. The purification from whole-cell extracts by chromatography on a Biohex70 ion exchange matrix and sucrose gradient sedimentation have been described previously (14, 15).

GST-VDR-LBD Affinity Binding Assay—The glutathione S-transferase-vitamin D receptor-ligand binding domain (amino acids 110–427) fusion protein (GST-VDR-LBD) was expressed in bacteria. Expressed fusion protein was extracted from the bacterial cells using standard procedures and passed through a Glutathione-agarose column (GST-agarose) (Pharmacia, Piscataway, New Jersey). The GST-agarose was washed with wash buffer [0.1 M KOAc in buffer H (20 mM Tris-OAc, pH 7.9, 1 mM EDTA) and 20% glycerol] and once with binding buffer (buffer H, 120 mM KOAc, 0.1% Nonidet P-40, 0.1 mM dithiothreitol, 0.2 mg/ml BSA, 0.1 mM phenylmethylsulfonyl fluoride). Binding reactions containing GST-VDR-LBD plus the protein fraction in a total volume of 400–800 μl were incubated with rotation.
Interaction of DRIP Coactivator and Pol II Holoenzyme

**FIG. 1.** DRIP complex copurifies with Pol II holoenzyme. HeLa whole-cell extracts were chromatographed on Biorex70, and eluted proteins were analyzed by immunoblotting for DRIP130 (top). The 0.6 M fraction was subjected to sucrose gradient sedimentation, and fractions were immunoblotted for DRIP130, DRIP150, and DRIP205 and the 210-kDa RPB1 subunit of Pol II, Cdk8, and Med7 (bottom). The Cdk8-specific band is indicated by the arrow. Fractions on the left (low numbers) represent rapidly sedimenting complexes, and fractions on the right (high numbers) represent low molecular weight complexes. L, load; FT, flow-through.

16 h at 4 °C in binding buffer containing 0.18 M KCl, 20 mM Tris-OAc, pH 7.9, 0.1% Nonidet P-40, 1 mM dithiothreitol, and 0.2 mg/ml BSA in the presence or absence of 0.5 μM 1,25(OH)2D3 (BioMol). The amount of total protein included in binding reactions was 560 μg when using nuclear extract or 260 or 540 μg when using sucrose gradient fractions 10 or 18, respectively. In Fig. 2, the effect of binding on different concentrations of 1,25(OH)2D3 was tested. After binding, the supernatant was removed, and protein beads were then washed three times using 400–800 μl of wash buffer (500 mM KCl, 20 mM Tris-OAc, pH 7.9, 0.1% Nonidet P-40, 1 mM dithiothreitol, 0.2 mg/ml BSA) in the presence (0.5 μM) or absence of 1,25(OH)2D3. For Western blot analysis, samples were subjected to electrophoresis in 5% SDS-polyacrylamide gels and immunoblotted using the indicated antibodies.

**Immunoprecipitation—**130 μg of protein from fraction 12 or 100 μg from fraction 2 of the sucrose gradient was immunoprecipitated with anti-DRIP130 antibody. Pol II in this fraction binds to GST-VDR-LBD in the presence of 1,25(OH)2D3 (not shown). 400–800 μl of binding reactions were incubated with rotation for overnight at 4 °C in buffer H supplemented with 0.1% Nonidet P-40, 0.1 mM dithiothreitol, 0.2 μg/ml BSA, and 1 mM phenylmethylsulfonyl fluoride in the presence of protein extract, 3–4.5 μl of antibody, and 10–20 μl of protein A beads. These steps were performed with and without 0.5 μM 1,25(OH)2D3. For Western blot analysis, samples were subjected to electrophoresis in 7% SDS-polyacrylamide gels and immunoblotted using the indicated antibodies. In the case of the polyclonal Cdk8 antisemur used in Fig. 3a, antibody molecules specific for the GST fusion partner were adsorbed out using a GST protein matrix.

**RESULTS**

**DRIP Complex Copurifies with Pol II Holoenzyme**—The DRIP complex, and the many similar SRB and mediator-containing complexes, were identified as key coactivator complexes for the regulation of transcription by multiple factors (reviewed in Ref. 16). We have developed a purification strategy for the Pol II holoenzyme (14, 15) and have tested which of the protein fractions in our standard purification contain DRIP polypeptides. HeLa whole-cell extracts were chromatographed on a Biorex70 ion exchange matrix, and protein was eluted in washes of increasing concentrations of potassium acetate. Immunoblotting of eluted proteins revealed the presence of DRIP130 only in the 0.6 M KOAc elution (Fig. 1, top). This protein fraction, which also contains the Pol II holoenzyme (14), was then subjected to sucrose gradient sedimentation in our standard purification protocol. Analysis of the sucrose gradient sedimentation fractions with available anti-DRIP antibodies revealed that DRIP130, DRIP150, and DRIP205 sediment in a broad peak from fractions 10–24. Although these proteins all cosediment, the fractions containing the highest concentrations of each were different, with DRIP130 having its highest concentration in fraction 18, DRIP150 in fraction 14, and DRIP205 in fraction 22 (Fig. 1, bottom). It is unknown whether there is a single composition for a single DRIP complex or, rather, a population of DRIP complexes with different compositions. The results of this sucrose sedimentation gradient support the latter possibility, because we observe different stoichiometries of DRIP factors among different fractions containing DRIP polypeptides. The meaning of this observation is unclear at this time. The Pol II holoenzyme peaked in fractions 10–18 in these same samples (Fig. 1, bottom). The different Pol II holoenzyme components did not perfectly cosediment in this gradient; this may reflect the multiple complexes in which these factors are present. For example, Med7 is present in the highest concentrations in fractions 10–14, consistent with the usual position of the Pol II holoenzyme on these gradients, but Med7 protein is present in low concentration in fractions up to number 24, possibly consistent with its presence in the DRIP complex (6). By comparison, Cdk8, which has not been detected in the DRIP or activator-recruited (ARC) complexes (9), was present only in fractions 10–18, consistent with the Pol II holoenzyme. These results demonstrate that on these initial purification steps, the DRIP complex subunits copurify with Pol II holoenzyme components. Because the DRIP complex and the Pol II holoenzyme share components such as Med7 and human RGR1/DRIP150, it is possible that a subset of the DRIP complex may interact directly with Pol II holoenzyme in a pre-formed complex. This possibility was tested by receptor binding experiments and by immunoprecipitation.

**Ligand-dependent Purification of Pol II Complexes by Vitamin D Receptor**—We examined whether an RNA Pol II complex associates with the VDR ligand binding domain using the GST-VDR-LBD bound to glutathione-agarose as an affinity matrix. Crude nuclear extract was incubated with GST-VDR-LBD in the presence of various concentrations of 1,25(OH)2D3, samples were washed in 0.5 mM KCl, and bound proteins were subjected to SDS-PAGE and assayed by Western blotting. As shown previously for the DRIP factors, the receptor plus ligand results in an association of the DRIP130 and DRIP205 polypeptides. Interestingly, the DRIP130 antibody detects three polypeptides, but the fastest migrating of these most efficiently interacts with the receptor (Fig. 2a). The large subunit of Pol II was purified on the GST-VDR-LBD matrix, and this was dependent upon the presence of 1,25(OH)2D3 (Fig. 2a). By contrast, BRCA1, another Pol II holoenzyme component (14), was not purified by the GST-VDR-LBD complex. This result suggests that the ligand bound GST-VDR-LBD interacts with the DRIP complex, which in turn interacts with Pol II, but if it is interacting with the holoenzyme version of Pol II, then it is associating with a subpopulation that does not contain BRCA1. As a control, liganded GST-VDR-LBD did not bind to purified core Pol II (data not shown), suggesting that the DRIP complex and/or Pol II holoenzyme components are necessary to bind liganded receptor.

**Differential Binding of Holoenzyme Complexes to the Vitamin D Receptor**—To test whether all Pol II complexes interact with the DRIP complex, we assayed binding to GST-VDR-LBD using different Pol II holoenzyme- and DRIP-containing protein fractions sedimented by sucrose gradient centrifugation. Vitamin D dependence for binding to the receptor was tested by immunostaining for Pol II and DRIP150 (Fig. 2b). DRIP150 is homologous to yeast RGR1, a component of the SRB/Med complex. The selected fractions were chosen to test the form of Pol II associating with the DRIP complex. These fractions have
high concentrations of DRIP205, which is the key subunit for anchoring DRIP to the vitamin D receptor in a ligand-dependent fashion (6). Fraction 10 contained the fastest sedimenting form of Pol II holoenzyme, which contains high concentrations of Med7 and Cdk8 (Fig. 1). Fraction 18 had lower concentrations of Med7 and Cdk8. Whether the Pol II in this latter fraction is core Pol II or a differentially sedimenting subpopulation of holoenzyme is unclear. In fraction 10, Pol II was purified by the GST-VDR-LBD matrix in the presence of 1,25(OH)2D3, but in fraction 18, Pol II was not purified. As a control for the affinity purification, DRIP150 was purified in both fractions (Fig. 2b). These data suggest that there are subpopulations of Pol II complexes that differentially associate with the vitamin D receptor via the DRIP complex.

Interestingly, as was seen in Fig. 2a for DRIP130, two bands were stained by the antibody specific for DRIP150, and only the lower band bound to the receptor (lanes 3, 6). The affinity purification by the receptor of the DRIP130 and DRIP150 polypeptides that migrate rapidly on SDS-PAGE may be because of phosphorylation of these subunits. Alternatively, other modifications such as acetylation, glycosylation, or even partial proteolysis could be responsible for shifting the migration of these polypeptides. It is noteworthy that DRIP205, which does not evidence a shift in migration (Fig. 2a), is the subunit that directly contacts the liganded VDR (6); this would suggest that DRIP complex binding to the liganded VDR may either induce an allosteric change, which is possible only with the rapidly migrating forms of DRIP130 and DRIP150, or that receptor binding selects a subcomplex from among the DRIP complexes.

Interaction of DRIP Complex with Pol II Holoenzyme Is Dependent upon Both 1,25(OH)2D3 and Vitamin D Receptor—The copurification of Pol II and DRIP complexes using conventional

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**Fig. 2.** Ligand-dependent purification of Pol II complexes by vitamin D receptor. a, crude nuclear extract was incubated with immobilized GST-VDR-LBD with 1,25(OH)2D3 present at 0, 0.5, 1.0, and 5.0 μM (lanes 2–5, respectively). Samples were washed in 0.5 M KCl, and bound proteins were subjected to 5% SDS-PAGE and immunoblotted for Pol II, BRCA1, DRIP130, and DRIP205. 10% of the input sample was analyzed in lane 1. b, binding to GST-VDR-LBD was assayed using different fractions containing Pol II and the DRIPs. Sucrose gradient fraction 10 (lanes 1–3) and fraction 18 (lanes 4–6) were incubated with GST-VDR-LBD in 0.5 μM 1,25(OH)2D3 (lanes 3 and 6), and bound proteins were subjected to 6% SDS-PAGE and immunoblotted for the large subunit of Pol II and for DRIP150. 10% of the input sample was analyzed in each of lanes 1 and 4.

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**Fig. 3.** Binding of DRIP complex to holoenzyme complex requires receptor plus ligand. a, immunopurification from sucrose gradient fraction 12 of Fig. 1 was performed using anti-DRIP130 antisera (lane 2–5). Anti-DRIP130 antibody, protein A beads, and the supernatant were stained by the antibody specific for DRIP150, and only the lower band bound to the receptor. 10% of the input sample was analyzed in lanes 1 and 4. 5% of the input sample was analyzed in lane 1. In., input; IP, immunoprecipitation. b, DRIP complex with low DRIP205 and DRIP150 content does not associate with Pol II holoenzyme. Immunopurification from sucrose gradient fraction 2 containing DRIP130 and Pol II but diminished DRIP205 and DRIP150 (see Fig. 1) was performed using anti-DRIP130 antibody as in panel a (lanes 2 and 3). Purified GST-VDR-LBD and 1,25(OH)2D3 (0.5 μM) was included in lanes 3. 5% of the input sample was analyzed in lane 1.

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**Fig. 4.** Model for function of the regulation of transcription by the VDR. Ligand binding to the VDR induces a structural change, exposing a binding surface for the DRIP205 subunit of the DRIP coactivator complex. Data presented in Figs. 2 and 3 suggest that binding of the DRIP205 to the liganded receptor induces a structural change in the DRIP complex that enables binding to the Pol II holoenzyme (HOLO) complex. The changes in shape and color for the VDR and the DRIP complex connote an allosteric change in the complex. RV, retinoid X receptor.
ated with the Pol II because Pol II was not immunopurified using the DRIP130 specific antibody (Fig. 3a, lane 2). Because Pol II was purified from this sucrose gradient fraction using GST-VDR-LBD matrix in the presence of the ligand, we tested whether association of a Pol II complex with the DRIP complex was dependent upon the presence of GST-VDR-LBD and ligand. Only when the purified GST-VDR-LBD and 1,25(OH)\textsubscript{2}D\textsubscript{3} were included in binding reactions did the polymerase complex associate with the DRIP complex (Fig. 3a, lane 5). In addition, the holoenzyme-specific Cdk8 polypeptide was purified under these conditions, demonstrating that it was the Pol II holoenzyme complex associating with the DRIP complex. Note that when in the presence of 1,25(OH)\textsubscript{2}D\textsubscript{3}, the Pol II and Cdk8 were present in immunoprecipitates above background levels (Fig. 3a, lane 4), probably because of low levels of contaminating VDR in the protein fractions used for these assays.

Because DRIP205 directly contacts liganded receptor (6), we tested whether DRIP205 was required for interaction with the Pol II holoenzyme. The sucrose gradient purification resulted in a very rapidly sedimenting complex containing Pol II and DRIP130 (fractions 2–4 in Fig. 1), and decreased DRIP205 and DRIP150. Immunoprecipitation with anti-DRIP130 antibody from fraction 2 did not purify Pol II from this complex (Fig. 3b, lanes 2 and 3). Upon prolonged exposure of this Western blot, a very faint Pol II band is detected in lane 3 (data not shown) in the presence of ligand and receptor, consistent with a low level of DRIP205 present in this protein fraction. This result suggests that interaction of the vitamin D receptor with DRIP205 is required for the DRIP complex to bind to the Pol II holoenzyme.

**DISCUSSION**

In these experiments we found that the DRIP coactivator complex and the Pol II holoenzyme copurify on initial purification steps. Because the DRIP complex contains holoenzyme components such as Med7 and RGR1/DRIP150, we tested whether this copurification might suggest association of the two complexes. Indeed, Pol II-containing complexes bind to the vitamin D receptor in a ligand-dependent manner, but immunopurification using antisera specific for DRIP130 reveals that the Pol II holoenzyme complex is not associated with the DRIP complex. Instead, Pol II holoenzyme only associated with the DRIP complex when in the presence of the receptor and ligand. This observation suggests that receptor plus ligand associate with the DRIP complex and that the receptor-ligand-DRIP complex together creates a binding surface for the Pol II holoenzyme. From these data we propose a model (Fig. 4) by which gene expression is stimulated by the ligand-bound vitamin D receptor bound to the promoter DNA. The DRIP complex will be recruited to the receptor, and together these facilitate the recruitment of the Pol II holoenzyme. It is known that the binding of the ligand to the receptor induces a change in the ligand binding domain structure (indicated by the change in shape and color of the receptor in Fig. 4; reviewed in Ref. 4). We suggest that the liganded receptor then binds to the DRIP complex and induces an allosteric change in DRIP structure (depicted as a change in color and shape), allowing it to bind to the Pol II holoenzyme and recruit the transcription machinery to the promoter. A formal possibility, which cannot now be excluded, is that both receptor and DRIP complex may independently contribute to binding to the Pol II holoenzyme, creating a stable interaction only when receptor is bound to the DRIP complex.

It has been suggested that the mediator complex associates with the core Pol II to constitute the Pol II holoenzyme (17). Data presented here argue against that model, at least as applies to the DRIP complex. The DRIP complex does not bind to all Pol II complexes because it did not bind to the Pol II in fraction 18 of the sucrose gradient (Fig. 2b), even though Pol II was quite abundant in the sample. A direct test of this hypothesis was not possible with our protein preparations because the DRIP complex was not separated from Pol II in any of the protein fractions. We suggest that the DRIP complex functions as a separable coactivator that interacts with the Pol II holoenzyme to drive gene expression.

The DRIP complex functions as a coactivator for transcription (5, 6, 9). Other coactivators known to function with nuclear receptors are the SRC/p160 class of factors. These different coactivators may have complementary activities, because the SRC coactivators, in conjunction with CBP/p300, appear to stimulate gene expression via the acetylation of genomic DNA adjacent to promoters and enhancers containing nuclear receptor binding sites (reviewed in Ref. 4). The data presented in this paper support a model in which the DRIP complex functions via an induced interaction with the Pol II holoenzyme. Thus, one coactivator would prepare the chromatin template, and the second would recruit the transcription machinery.

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