Isolation and Characterization of a Novel Ligand-dependent Thyroid Hormone Receptor-coactivating Protein*

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The thyroid hormone receptor (TR),1 which is a member of the steroid/thyroid receptor superfamily, regulates the expression of many target genes upon binding to triiodothyronine (T3) response elements. In the presence of T3, the TR recruits coactivating proteins that both modulate and integrate the ligand response. We report here the cloning of a novel protein using the TR ligand-binding domain as bait in the yeast two-hybrid system. Analysis of a putative full-length clone demonstrates a cDNA sequence that encodes a protein of 920 amino acids with a size of 120 kDa (p120). Alignment with known sequences shows homology to a previously identified protein of unknown function, termed skeletal muscle abundant protein. Interaction studies demonstrate that p120 interacts with the TR AF-2 domain in the presence of ligand through a 111-amino acid region. Northern analysis demonstrates widespread expression in human tissues. Cotransfection assays in CV-1 cells demonstrate that p120 enhances TR-mediated transactivation on multiple T3 response elements in the presence of T3. In addition, CREB-binding protein synergizes with p120 to enhance this effect. When linked to the GAL4 DNA-binding domain, p120 is an activator of transcription alone. Thus, p120 satisfies a number of important criteria as a nuclear receptor coactivator.

TR hinge region (7–10). In the presence of ligand, these corepressors are released from the TR, and proteins termed coactivators are recruited to mediate the ligand-dependent response (11). Although several nuclear receptor-interacting or -coactivating proteins such as SRC-1, RIP140, TIF2 (GRIP1), and TRIP1 have been isolated, their mechanism of action and physiologic role remain unclear (12–17).

Nuclear receptors, including the TR, have two distinct transcriptional activation domains, an AF-1 domain in the N terminus (18–20) and an AF-2 domain in the extreme C terminus, a well conserved region among nuclear receptors (NRs) that is believed to be essential for ligand-dependent transcriptional activation (21–23). Recently, x-ray crystallographic studies of the rat TRα1 ligand-binding domain revealed that a conformational change in the hormone-binding cavity is formed, exposing the AF-2 region when ligand binds to the receptor (24). Mutation of the AF-2 region of the TR causes the receptor to lose its ability to activate transcription despite its ability to bind both ligand and DNA, suggesting that TR-associated coactivators require an intact AF-2 region to interact with the TR (25, 26). Thus, putative coactivators should interact with the TR AF-2 domain and be able to enhance ligand-dependent stimulation. The recently identified coactivators SRC-1, TIF2, and RIP140 all appear to bind the NR AF-2 domain, although one report suggests that SRC-1 may interact with other regions of the TR (12, 14, 27).

Once bound to the coactivator, it is likely that other proteins are necessary for the TR to mediate transcriptional enhancement. Recently, it has been reported that activation by NRs can be enhanced by the CREB-binding protein-binding protein (CBP/p300) through a direct ligand-dependent interaction (13). Furthermore, CBP/p300 also interacts with SRC-1, suggesting that CBP/p300 may play a role as an integrator of NR-mediated activation of gene expression (28–30) by signaling RNA polymerase II or by acting to modify histone structure through an intrinsic histone acetyltransferase activity (31).

While a number of possible proteins that mediate the response to thyroid hormone have been identified, it is likely that tissue-specific responses will depend on the existence of other coactivators whose regulation in certain tissues may be paramount in mediating the T3 response. To address this issue further, we have used the yeast two-hybrid system to isolate TR-interacting proteins (32). We have isolated a novel protein (p120) that contains a bromodomain in its C terminus and that interacts with the TR in a ligand-dependent manner both in yeast and in vitro. This ligand-dependent interaction is dependent upon an intact AF-2 domain. Furthermore, cotransfection studies in mammalian cells demonstrate that p120 enhances the AF-2 function of the TR. When linked to a heterologous DNA-binding domain, p120 is a significant activator of transcription. Taken together, these data indicate that p120 is a nuclear receptor coactivator. Further studies will be needed to address its physiologic role.

1 The abbreviations used are: TR, thyroid hormone receptor; TRE, thyroid hormone response element; T3, triiodothyronine; SRC, steroid receptor coactivator; RIP, receptor-interacting protein; TIF, transcriptional intermediary factor; AF-2, activation function-2; NR, nuclear receptor; CBP, CREB-binding protein; bp, base pair(s); kb, kilobase pair(s); GST, glutathione S-transferase; RAR, retinoic acid receptor; ER, estrogen receptor; AR, androgen receptor; EMSA, electrophoretic mobility shift assay; SMAP, skeletal muscle abundant protein; TRIP, thyroid receptor-interacting protein.

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Materials and Methods

Yeast Two-hybrid System—A PstI-PstI cDNA fragment encoding amino acids 204–461 of the human TRβ1 isoform was ligated to the EcoRI-PstI sites of pGBT9 (CLONTECH) using an EcoRI-PstI-BamHI adaptor. The yeast strain HF7c was cotransfected with the GAL4-TRβ1 construct and a human placenta cDNA library in pGAD10 (CLONTECH) as described previously (9). Transformants were plated onto synthetic dropout media plates lacking histidine, leucine, and tryptophan. Viable colonies were dissolved in 40 μl of Z buffer (0.06 M NaHPO4, 0.04 M Na2HPO4, 1 mM MgSO4, and 10 mM KCl). The colonies were then frozen in liquid nitrogen for 10 s and thawed at room temperature. This freeze-thaw step was repeated again. β-Galactosidase activity in yeast cells was measured by a chemiluminescent assay (Tropix Inc.). Identification of positive clones was performed by culturing His− Lac− transformant yeast colonies in 3 ml of liquid synthetic medium (without Trp and Leu) in the presence or absence of 10 μM T3. For analysis of the interaction of p120 constructs with TRβ1, a sense oligonucleotide, p120 START (5′-GGCGGAATTCTCTTCGTGCA-3′) and the pKCR-end primer (5′-TAACATTATAAGCTGTTATA-3′) in the antisense orientation were used to amplify the entire p120 cDNA and, at the same time, create a unique EcoRI site at the 5′ terminus. The polymerase chain reaction product was digested with Asp-718, blunt-ended with Klenow polymerase, and then digested with EcoRI. The p120 cDNA was ligated into pGAD24 that had been digested with EcoRI and SmaI. p120 deletion mutants were created by using unique restriction sites and then ligated into pGAD10 or pGAD24 in frame.

Restrictions were studied in the yeast strain HF7c as described above by transforming with the appropriate pGBT9 and pGAD24 constructs. Transformants were plated onto synthetic complete media plates lacking histidine, leucine, and tryptophan that either lacked ligand or contained 1 μM T3. After 3–4 days, viable colonies were dissolved in 40 μl of Z buffer. The colonies were then frozen in liquid nitrogen for 10 s and thawed at room temperature. This freeze-thaw step was repeated again. β-Galactosidase activity was measured by the chemiluminescence assay. Each point is the result of between two and three independent experiments. The data are the pooled results ± S.E.

Screening of a Human Fetal Brain Library—The Uni-ZAP XR fetal brain cDNA library ( Stratagene) was screened with the original 900-bp insert of pGAD10-p120 labeled with [32P]dCTP by random priming under high stringency conditions. The largest hybridizing clone contained sequences 5′- and 3′-ends; however, one of the clones identified was TRIP1, human RAR (a gift of W. W. Chin (14)) and rat CBP (a gift of R. H. Goodman (35)) were also placed into pKCR.

UAS-TKLuc contains five copies of the 17-bp upstream activating sequence upstream of TK-Luc, and the GAL4 DNA-binding domain is in the pG5 vector (36). The p120 constructs (amino acids 1–920 and 1–297) were amplified by polymerase chain reaction and placed in frame with the GAL4 DNA-binding domain and the VP-16 activation domain linked to the GAL4 DNA-binding domain. All plasmids for transfection were prepared by column purification (QIA-GEN Inc.) and were generally subjected to two separate plasmid preparations.

Cell Culture and Transient Transfection—CV-1 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 μg/ml penicillin, 0.25 μg/ml streptomycin, and amphotericin. Transient transfections were performed using the calcium phosphate technique in six-well plates with each well, unless otherwise indicated, receiving 1.7 μg of reporter, 20 ng of expression vector, and 500 ng of pKCR alone or with an equal amount of pKCR-p120 or pKCR-SRC-1. Each well also received 20 ng of a cytomegalovirus-driven β-galactosidase expression vector to control for transfection efficiency. 18–20 h after transfection, the cells were washed with phosphate-buffered saline, and the medium was changed to Dulbecco’s modified Eagle’s medium with 10% steroid hormone-depleted fetal bovine serum supplemented with 10 ng/ml T3. To remove steroid hormones and thyroid hormones, fetal bovine serum was treated with 50 mg/ml actinomycin D (Sigma) and 30 mg/ml exchange resin (type AG X8, analytical grade, Bio-Rad). 40–44 h after transfection, the cells were harvested in extraction buffer and assayed for both luciferase and β-galactosidase activities (Tropix Inc.). Luciferase activities were corrected for β-galactosidase activity as indicated. All experiments were performed in triplicate and repeated at least three times. The data are the pooled results ± S.E.

Electrophoretic Mobility Shift Assays (EMSAs)—EMSAs was carried out using 3–4 μl of in vitro translated nuclear receptors from rabbit reticulocyte lysate (Promega). GST-p120-186–297 and GST alone were made as described above and eluted from glutathione-agarose beads in a Tris/glutathione buffer. 15 μg of GST-p120 or GST alone were used in the EMSAs. The proteins were incubated with 50,000–75,000 cpm of a 32P-radioabeled probe in the presence of salmon sperm, poly-deoxy-inosin-deoxy-cytidylic acid, and dithiothreitol for 20 min at room temperature. Complexes were resolved on 5% nondenaturing acrylamide gels followed by autoradiography. The concentration of T3 used was 100 nM.

Accession Number—The GenBank™ accession number for the cDNA sequence of p120 is AF016270.

Results

Identification of the TR-Associated Protein p120—To identify proteins that interact with the TR, we employed the yeast two-hybrid system. The hinge region and the ligand-binding domain of human TRβ1 (amino acids 204–461) were fused to the GAL4 DNA-binding domain in the vector pGBT9. This GAL4 DNA-binding domain–TR construct was used to screen a human placenta cDNA library fused to the GAL4 activation domain. We identified a number of positive clones, two of which showed a significant enhancement of interaction in the presence of T3 (Fig. 1). One of these clones was identified as TRP1, which has been previously demonstrated to interact with the TR in yeast and mammalian cells (17). Sequence analysis of the other showed a 900-bp clone that appeared to be a portion of a previously identified clone termed skeletal muscle abundant protein (SMAP, GenBank™/EMBL accession number X87613), which was identified randomly from a Jurkat cell library and felt to be a transcription factor based on the presence of a bromodomain in its C terminus (37).

To gain a full-length version of this protein, a human fetal brain cDNA library was screened using the cDNA recovered from the yeast two-hybrid screen. Two positive clones were identified. Both have similar 5′- and 3′-ends; however, one of the clones contains an internal deletion and is currently being evaluated. The other clone was found to be 3154 bp and contain an open reading frame encoding a protein of 920 amino acids (Fig. 2). While the coding and amino acid sequences are similar to that of SMAP, an insertion of 5 nucleotides in the
putative 5′-untranslated region of SMAP leads to the addition of 116 amino acids to the open reading frame such that homology to the 5′-end of SMAP begins at amino acid 117. A second insertion leads to a 40-amino acid insertion at amino acid 304, and a third insertion of 15 amino acids is present at amino acid 887 of this novel clone, which is not present in SMAP. Otherwise, the two proteins are identical. The relationship between this protein and SMAP is depicted in Fig. 2A. The predicted molecular mass of this novel protein is 100 kDa, and that achieved with in vitro translation is 120 kDa; and we have termed the protein p120. The protein contains a number of putative nuclear localization signals present in the amino terminus and a conserved bromodomain between amino acids 772 and 857 (38). Furthermore, there is remote amino acid homology (14%) between amino acids 254–743 of p120 and amino acids 426–916 of TIF2.

The initial placental clone isolated in the yeast two-hybrid screen contains an additional 21 bp 5′ to the cDNA sequence isolated from the human fetal brain library. An in-frame ATG codon is present, but we could not identify this sequence in additional library screening. Interestingly, a mouse-expressed sequence tag (ma33eo1.r1) from brain of 294 bp, which is nearly homologous to p120, also contains a 20-bp extension from the cDNA identified. However, this extension is not homologous to the human sequence. Thus, the significance of further 5′-sequences remains to be determined.

p120 Is Expressed Ubiquitously in Human Tissues—To determine whether p120 is expressed only in certain tissues, we performed Northern analysis on multiple human tissues using a probe encompassing the first 800 bp of the p120 cDNA sequence. Our results, shown in Fig. 3, are consistent with those of Nielsen et al. (37) from their analysis of SMAP expression and demonstrate widespread expression of a 3.5-kb isoform and more restricted expression of a larger isoform of 4.8 kb that is predominantly expressed in the placenta, liver, and pancreas. We also performed a similar analysis on a variety of human cell lines and found that the 3.5-kb species was present in all cell lines examined (data not shown). Thus, like other coactivators, p120 appears to be expressed ubiquitously.

p120 Interacts with the TR AF-2 Domain through a Specific Interacting Domain—As the initial yeast clone contained the first 297 amino acids of p120, we chose to examine this region for a specific TR-interacting domain. We inserted the full-length p120 molecule and a number of its derivatives in frame with the GAL4 activation domain in vector pGAD24. Interactions were examined in yeast with the TR C terminus or TR mutants fused to the GAL4 DNA-binding domain. In the absence of ligand, each of the p120 constructs containing amino acids 186–297 had very little ability to interact with the TR C terminus (Fig. 4A). The addition of T3 allowed the full-length construct and other constructs that contained amino acids 186–297 to interact strongly with the TR. Surprisingly, amino acids 1–187 alone have a significant ligand-independent ability to interact with the TR. These data suggest that amino acids 186–297 act as an interacting switch to promote interaction in the presence of ligand. Analysis of the sequence in this region of p120 shows a consensus NR box (LSELL, amino acids 240–244) that appears to be essential for mediating interactions with the nuclear hormone receptors and that is present in most of the other identified coactivating proteins (39, 40). A second such motif exists outside of the identified interacting domain (amino acids 309–313), and its function has not been ascertained.

To confirm these observations, we performed similar experiments in vitro with bacterially expressed GST-p120 deriva-

Fig. 2. Sequence analysis of p120 demonstrates significant homology to SMAP. A human fetal brain library was screened with the original clone isolated from the yeast two-hybrid screen, and two separate clones were identified with similar 5′- and 3′-ends. A, the 5′-cDNA end is identical to that of SMAP (37), except for a 5-base pair insertion at base pair 414, which leads to a 116-amino acid insertion in the amino terminus. Amino acid homology to SMAP begins at amino acid 117. A second insertion leads to a 40-amino acid insertion at amino acid 304. A third insertion of 15 amino acids is present at amino acid 887 of this novel clone, which is not present in SMAP. The bromodomain between amino acids 772 and 857 is black, whereas the TR-interacting domain is gray. B, the amino acid sequence of p120 is shown. The TR-interacting domain between amino acids 186 and 297 is boxed, and the bromodomain is underlined. A number of putative nuclear localization signals are present in the first 300 amino acids of the protein.
tives. As Fig. 4B demonstrates, amino acids 1–297 promote a strong ligand-dependent interaction (lanes 2 and 3), whereas deletion of amino acids 186–297 causes p120 to bind the TR in a ligand-independent fashion only (lanes 4 and 5), similar to what was found in yeast. Finally, GST-p120-(186–297) was able to function independently to promote a very strong ligand-dependent interaction with the TR (lanes 6 and 7). Taken together, these data confirm the importance of a small region of p120 in being responsible for the ligand-dependent association between the TR and p120.

We next examined the ability of the TR AF-2 mutant to interact with p120. We used the E457A mutant, which has been demonstrated to bind ligand normally yet be transcriptionally inactive (21). In the yeast two-hybrid system, there was very little interaction between TR E457A and the amino-terminal portion of p120 (amino acids 1–297) as shown in Fig. 5A. Similar data were found using GST-p120-(1–297), where no evidence of a ligand-dependent interaction was seen (Fig. 5B). To confirm that these solution assays were in agreement with DNA binding studies, we performed EMSA with the p120 interacting domain and in vitro translated wild-type TR and TR E457A. As Fig. 5C demonstrates, the p120 fragment bound to the wild-type TR homodimer and heterodimer in the presence of T3 only. In contrast, the TR AF-2 mutant could not bind to p120 on this DR+4 element despite binding ligand normally. Thus, in agreement with the principle of coactivation, an intact AF-2 domain is essential for the TR to interact with p120. Further analysis of the domains of the TR necessary for interacting with p120 was also performed both in yeast and using GST fusion proteins. Our data demonstrate that an intact hinge region, which is necessary for binding corepressor proteins, is not necessary to bind p120 (data not shown).

p120 Acts to Enhance Thyroid Hormone-dependent Activation of Reporter Expression—Because p120 interacts strongly with the TR in the presence of ligand, we next examined its ability to functionally affect T3-mediated activation of gene expression. We cotransfected CV-1 cells with three separate positive TREs upstream of the TK109 promoter linked to luciferase. In each case, excess cotransfected p120 was able to augment ligand-dependent activation of reporter expression by 2–3-fold both in terms of overall luciferase expression and -fold activation over basal levels (Fig. 6A). p120 had little effect on basal activity (in the absence of T3) with each of the three reporters studied. The parental vector containing the TK109 promoter alone was not affected by cotransfected p120. Similar data were also obtained with single palindromic and DR-4 TREs linked to the SV40 promoter (data not shown). Thus, cotransfected p120 is able to function efficiently when cotransfected in excess in mammalian cells, and its effect is promoter-independent.

As p120 interacts with the AF-2 region of the TR, we next chose to examine the ability of p120 to augment transcription of a number of TR mutants on the palindromic TRE. Mutation of the AF-2 domain (E457A) significantly impaired the ability of this receptor to respond to T3 (Fig. 6B) both in the presence and absence of p120. However, p120 did augment the function of the E457A mutant, although not to the level of the wild-type receptor, consistent with an impaired interaction. These data are similar to those of Collingwood et al. (27) using SRC-1 to augment the function of a TR AF-2 mutant on a palindromic TRE. The hinge mutant, P214R (41), responded well to T3 both in the presence and absence of p120. Its -fold activation was impaired principally because it does not repress transcription in the absence of ligand. L428R, a mutation of the ninth heptad (42), caused a loss of ligand binding and thus impaired any functional interaction with p120.

We next chose to compare p120 with SRC-1 and TRIP1 in an identical transfection paradigm. As shown in Fig. 6C, p120 and F-SRC-1 have similar coactivation profiles on the palindromic TRE and DR-4 reporters in that there is a 2-fold enhancement of overall luciferase activity and a similar enhancement in -fold induction. In contrast, TRIP1 is an inhibitor of T3-driven activation on both of the TREs studied, which is consistent with the
original report describing TRIP1 as a TR-interacting protein (17). Thus, p120 appears to be quite similar to SRC-1 in mediating transcriptional coactivation on the palindromic and DR+4 TREs in the transfection system used in these studies, despite their different structures. TRIP1 appears to function as a dominant inhibitor of endogenous coactivators, by perhaps interfering with available AF-2-binding sites.

p120 Is an Efficient Coactivator of Other Nuclear Receptors—As a number of identified putative coactivators appear to display an ability to functionally and structurally interact with multiple nuclear receptors, we next asked whether p120 could augment transcriptional activation by other nuclear receptors. The AR was most efficiently activated by p120 in the presence of its ligand, showing up to a 5-fold enhancement (Fig. 7) of overall luciferase activity. The ER was not well activated by p120, and the effects on the RARα isoform were very minor. Both the ER and RAR were tested on their respective response elements in the context of the thymidine kinase promoter, whereas the AR was tested on the murine mammary tumor virus promoter. Furthermore, we tested the ability of p120 to activate the acidic activator VP-16 when linked to the GAL4 DNA-binding domain and found that no activation occurred on a UAS-TKLuc reporter, whereas GAL4-TR was efficiently co-activated in the presence of ligand (data not shown). Thus, p120 appears to exert some specificity in its ability to function as a coactivator in the context of both steroid receptors and other transcriptional activators.

p120 Activates Transcription in CV-1 Cells through Amino Acids 297–920 when Fused to a Heterologous DNA-binding Domain—We next tested whether full-length p120 could activate transcription when linked to the GAL4 DNA-binding domain and tested on a reporter containing five UAS-binding sites (UAS-TKLuc). As shown in Fig. 8, GAL4-p120 activates reporter expression by 6–8-fold in CV-1 cells depending upon the amount of plasmid used. When amino acids 298–920 were deleted, activation was lost, suggesting that the C-terminal region contains an independent activation domain. Thus, when p120 is recruited near the proximal promoter, it is capable of enhancing transcription through a region contained between amino acids 298 and 920.

CBP and p120 Synergize to Enhance Thyroid Hormone Action—As CBP/p300 interacts with one of the known coactivators, SRC-1, we chose to examine the ability of p120 to synergize with coexpressed CBP in our transient transfection system. Interestingly, expression of increasing amounts of pKCR2-CBP had little effect on ligand-dependent activation by the TR, whereas p120 itself increased T3-dependent activation of the palindromic reporter by 2–3-fold (Fig. 9). However, coexpression of CBP and p120 further increased ligand-dependent reporter expression by 30–40%. Similar data were achieved on the palindromic reporter with SRC-1 as shown in Fig. 9. CBP had no effect on the ligand-independent activity of the TR alone or in the presence of p120 or SRC-1.

**DISCUSSION**

Ligand-dependent transcriptional activation by the thyroid hormone receptor involves at least two separate processes. Initially, the presence of ligand causes the TR-retinoid X receptor heterodimer or TR-TR homodimer to release members of the nuclear corepressor family that are responsible for dictating the degree of ligand-independent repression present (11). In the presence of ligand, the TR can then recruit coactivators or integrators, which then help mediate transcriptional activation by still undefined mechanisms. A number of interacting proteins for members of the nuclear receptor superfamily have been described. They include SRC-1, TIF2, RIP140, TRIP1, and ARA-70 (reviewed in Ref. 11). Each of these proteins shares the characteristic of interacting with NRs principally in the presence of ligand. Furthermore, a number of these proteins are able to functionally enhance ligand-dependent transcriptional activation as well as function as independent activators when brought to the proximal promoter by a heterologous DNA-
Here, we describe the molecular cloning of a cDNA encoding a novel protein of 920 amino acids with a molecular mass of 120 kDa, termed p120 (nuclear receptor coactivator). p120 fulfills each of the above criteria of coactivators and provides an interesting tool for the study of the mechanisms by which coactivators interact and mediate their effects.

In this report, we have demonstrated that p120 interacts with the TR through a specific 111-amino acid region present in the N terminus of the molecule. Our data in vivo in yeast and in vitro using bacterially expressed derivatives of p120 demonstrate that this region appears to act as a switch that allows the interaction to occur in the presence of T3. Deletion of this region allows the remaining N terminus to constitutively bind the TR. We hypothesize that this 111-amino acid region allows the TR to access the remaining N terminus in the presence of ligand through the initially exposed AF-2 domain. While our data do not rule out the presence of a second ligand-dependent domain in the C terminus, the strength and quality of the interaction in each of the systems employed suggest that this amino-terminal region is the principal interacting domain. The specificity of the interacting domain of p120 may underlie the selective nature of functional interactions between p120 and other nuclear receptors such that this ligand switch may permit only certain receptors to contact p120. It is clear from a number of separate groups that the motif LXXLL appears to mediate interactions with the nuclear hormone receptors. Such domains are present either as single or multiple copies in TIF1, RIP140, SRC-1, TIF2, CBP, p300, and a number of the TRIP proteins (39, 40, 43–45). While such a motif is present between amino acids 240 and 244 of p120, its function in interactions with other nuclear receptors has not been determined. However, functional assays suggest that this p120 interacting domain does not interact

![Figure 6](http://www.jbc.org/content/2837/7/29839/F6)

**FIG. 6.** Cotransfected p120 acts to functionally enhance thyroid hormone action in mammalian cells. A, CV-1 cells were cotransfected with the indicated TRE reporter or the parental TK109 reporter, TRβ1, either empty expression vector or equal amounts of expression vector containing p120, and a β-galactosidase expression vector in the presence or absence of 100 nM T3. The data are expressed as relative luciferase (LUC) activity, where 1 equals the activity of the reporter in the absence of ligand and p120. Fold (X) activation by T3 is also indicated over each bar. B, an identical paradigm was used on the palindromic TRE with a number of TR mutants. The data are quantified in an identical fashion as described for A. C, an identical paradigm was used on the palindromic TRE with a number of TR mutants, and the results are displayed as described above. pKCR2 indicates the TR and ligand effect in the presence of empty expression vector. PAL, palindromic TRE; WT, wild type.

![Figure 7](http://www.jbc.org/content/2837/7/29839/F7)

**Fig. 7.** Cotransfected p120 acts to enhance the activity of other nuclear receptors. CV-1 cells were transfected as described under “Materials and Methods,” with MMTV-Luc and the AR, ERα2-TK-Luc and the ER, or RAREα3-TK-Luc and RARα in the presence or absence of p120 and the respective ligands for each of the nuclear receptors (10−8 M dihydrotestosterone, 10−8 M estradiol, and 10−6 M 9-cis-retinoic acid). The data are quantified as relative luciferase (LUC) activity, where 1 equals the activity of the reporter in the absence of ligand and p120, with -fold (X) activity shown over each bar.

![Figure 8](http://www.jbc.org/content/2837/7/29839/F8)

**FIG. 8.** p120 contains an autonomous activation domain in the C-terminal portion of the protein. CV-1 cells were transfected with a UAS-TK-Luc reporter, increasing amounts (0.17, 0.83, and 1.6 μg) of the GAL4 DNA-binding domain alone or linked to full-length p120 or the amino-terminal interacting domain (amino acids 1–297), and a β-galactosidase expression vector. The data are quantified as -fold induction over the basal state in the presence of the GAL4 DNA-binding domain alone.
well with the RAR or the ER, suggesting that other amino acids within the interacting domain are important for determining receptor specificity. Further work will be needed to define the unique ligand switch present in p120 and to characterize its interactions with other nuclear receptors.

While a ligand-dependent interaction is necessary for coactivation, functional enhancement should be seen in cotransfection experiments. The coactivators SRC-1, TIF2, RIP140, and ARA-70 have all been described to enhance hormone-dependent gene transcription. In contrast, TRIP1 functions to inhibit TR-mediated activation. In our study, as in others, a significant excess of cotransfected coactivator must be used to achieve enhancement of Reporter activity. This may be due to the presence of high endogenous levels of p120, as demonstrated by Northern analysis (data not shown), and other such proteins that makes any increase in activation difficult to achieve. Furthermore, proteins necessary for p120 activity may be present also in a limited quantity and thus prevent significant enhancement. Thus, to properly judge the effectiveness of p120, we used similar amounts of SRC-1 and TRIP1 in the identical cotransfection paradigm. p120 is as effective in our system as SRC-1, whereas TRIP1, as described previously (17), acted to inhibit activation. p120 preferred to functionally interact with the TR and the AR, which may relate to its interacting domain. Furthermore, p120 was not a promiscuous activator as it was functionally inert in the presence of the VP-16 activation domain.

Preliminary mapping studies, performed in the context of a heterologous DNA-binding domain, demonstrate that amino acids 298–920 of p120 are responsible for full transcriptional activation in this setting. The region between amino acids 298 and 920 contains a well conserved bromodomain between amino acids 772 and 857. While the function of the bromodomain remains unclear, it is present in a number of transcriptional regulatory proteins (38). To date, only two other nuclear receptor-interacting proteins, TIF1 and CBP/p300, have been found to contain a bromodomain (44). In contrast to p120, TIF1 is an inhibitor of nuclear receptor activation; thus, the bromodomain in isolation may not be responsible for the independent activation function of p120. p120 may contain a novel activation domain that contacts other proteins or members of the basal transcriptional machinery to achieve coactivation. Our data demonstrate that p120 can synergize with CBP and perhaps help recruit it to the transcriptional complex in a similar fashion to SRC-1 (13, 28–30). Further work will need to be done to determine how p120 and CBP interact. Interestingly, the area of weak homology between p120 and TIF2 encompasses part of the homologous region in murine SRC-1 (amino acids 789–993) that is known to interact with CBP.

Our Northern analysis suggests that two isoforms of p120 may be expressed. To date, we have not been able to identify any further sequence 5’ to that presented in this report. We have identified a second clone that contains an internal deletion in the region of the interacting domain of p120. It is currently undergoing complete evaluation. The original SMAP clone identified by Nielsen et al. (37) is substantially smaller than p120 due to nucleotide differences. SMAP, however, still would contain the TR-interacting domain and the bromodomain in the C terminus. We hypothesize that p120 is a member of a novel family of proteins of which at least two other isoforms exist. Further work will need to address their functional properties as well as the tissue-specific expression of each of these proteins.

While a number of separate coactivators have been described, specificity and function in vivo have yet to be addressed. p120 is ubiquitously expressed and shares with SRC-1 the ability to coactivate TR-mediated activation of gene expression in the presence of ligand. This ability is dependent upon an intact AF-2 domain within the TR, which is necessary for binding in the presence of DNA. It is likely that a variety of coactivators can interact in vivo with the TR depending on the tissue of interest and the actual level of ligand. However, unlike SRC-1, p120 cannot coactivate the ER or RAR response (12). In contrast, TIF2 cannot enhance TR- or RAR-mediated transactivation, but functionally enhances AR-, progesterone receptor-, and ER-mediated action (16). Thus, despite similarities in structure, each of the identified coactivators appears to allow for specificity in mediating hormone-dependent transcription. Further work will be necessary to examine specificity both in vitro and in vivo.

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FIG. 9. CBP and p120 synergize to enhance thyroid hormone action. CV-1 cells were transiently transfected as described under "Materials and Methods" with identical amounts of either p120 or SRC-1, except that 170 ng of pKCR-CBP or pKCR, alone were also cotransfected. The results are shown as relative luciferase (LUC) activity, with 1 representing the activity of the palindromic reporter (PAL) in the absence of ligand. The concentration of T3 used was 100 nM. The results shown are the means ± S.E. of three separate experiments performed in triplicate.
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Tsuyoshi Monden, Fredric E. Wondisford and Anthony N. Hollenberg

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