Identification and Characterization of RRM-containing Coactivator Activator (CoAA) as TRBP-interacting Protein, and Its Splice Variant as a Coactivator Modulator (CoAM) *

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We previously cloned and characterized thyroid hormone receptor-binding protein (TRBP) as an LXXLL-containing general coactivator that associates with coactivator complexes through its C terminus. To identify protein cofactors for TRBP action, a Sos-Ras yeast two-hybrid cDNA library was screened using TRBP C terminus as bait. A novel coactivator was isolated, coactivator activator (CoAA), that specifically associates with TRBP. Human CoAA is composed of 669 amino acids with a TRBP-interacting domain and two highly conserved RNA recognition motifs (RRM) commonly found in ribonucleoproteins. A splice variant lacking the entire TRBP-interacting domain was also isolated as a coactivator modulator (CoAM), a 156-amino acid protein containing only the RRM region. Human CoAA and CoAM mRNAs are encoded by a single gene located on chromosome 11q13; alternative splicing in exon 2 of CoAA yields CoAM. CoAA interacts with both TRBP and p300 in vitro. In addition, CoAA potently coactivates transcription mediated by multiple hormone-response elements and acts synergistically with TRBP and CREB-binding protein (CBP). Furthermore, CoAA is associated with the DNA-dependent protein kinase-poly(ADP-ribose) polymerase complex. Strikingly, CoAM, which lacks a TRBP-interacting domain, strongly represses both TRBP and CBP action suggesting that CoAM may modulate endogenous CoAA function. These data suggest that CoAA may serve as a mediator of coactivators such as TRBP in gene activation.

Hormone-induced gene activation plays a central role in key biological phenomena such as cell proliferation, differentiation, apoptosis, and early development. Liganded nuclear receptors participate in a number of these processes by interacting with specific hormone response elements as well as transcriptional cofactors and permitting polymerase II complex to access target genes. A number of coactivators have been isolated and characterized. These include the general cointegrator CBP/CoAM, coactivator modulator; RNP, ribonucleoprotein; hnRNP, heterogeneous nuclear ribonucleoproteins; RRM, RNA-recognition motif; PGC-1, PPARγ-coactivator-1; DNA-PK, DNA-dependent protein kinase; PARF, poly(ADP-ribose) polymerase; pol II, RNA polymerase II; MEKK, MEK kinase; MMTV, mouse mammary tumor virus; PAGE, polyacrylamide gel electrophoresis; aa, amino acids; GR, glucocorticoid receptor; GRE, glucocorticoid response element; SRC-1, steroid receptor coactivator-1; CARM1, coactivator-associated arginine methyltransferase 1; SRA, steroid receptor RNA activator.

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† The abbreviations used are: CBP, CREB-binding protein; TRBP, thyroid hormone receptor-binding protein; CoAA, coactivator activator;
On the other hand, mRNA synthesis and mRNA processing are colocalized and are apparently functionally coupled within the same transcription complex, which is composed largely of nuclear RNAs that associate with nuclear ribonucleoproteins (RNPs) (21–23). The distribution of nuclear RNPs has recently been shown to play an important role in transcription. Thus, identification of the components of the transcriptional machinery that are responsible for the action of coactivators is critical.

Our efforts have focused on coactivator function and have taken advantage of our work with the general coactivator TRBP. TRBP is a high molecular weight (2063 amino acids) ubiquitously expressed coactivator (8–12). Although devoid of intrinsic histone acetyltransferase activity, TRBP can recruit this activity by associating with CBP/p300 (8, 9, 12). TRBP not only enhances nuclear receptor-mediated transcription but also stimulates the activities of multiple transcriptional factors including AP-1 and NFkB (8, 9).

EXPERIMENTAL PROCEDURES

**Plasmids—** Mouse CBP, p300, human TRBP and its derived plasmids have been described previously (8). Full-length human CoAA and CoAM were generated by PCR and inserted into the same transcription complex, which is composed largely of nuclear DNA-dependent protein kinase (DNA-PK) and poly(ADP-ribose) polymerase (PARP) complexes that are implicated in transcription regulation (8). These data suggest that TRBP might be targeted to the transcriptional complex via its C terminus. Thus, we searched for TRBP-interacting proteins by a yeast two-hybrid screen using TRBP C terminus as bait.

We report here the isolation and characterization of a coactivator activator (CoAA) and its splice variant coactivator modulator (CoAM). CoAA stimulates transcription through its interactions with TRBP and RNA-containing transcriptional complexes. CoAM, which is unable to interact with TRBP, competes with CoAA and modulates CoAA function. Both CoAA and CoAM contain conserved RNA recognition motifs (RRMs) and thus may belong to the RNP family, which structurally and functionally may serve as integral components of the transcriptional machinery.

**RESULTS**

**Cloning of CoAA and CoAM—** The nuclear receptor coactivator TRBP has been shown previously to be a potent general coactivator that stimulates transcription mediated by multiple hormone-response elements. Further evidence suggested that the C terminus of TRBP may interact with protein complexes involved in the transcription machinery. In order to identify protein factors that directly associate with TRBP, a rat pituitary cDNA library was screened with a modified Sos-Ras yeast two-hybrid system using the C terminus of TRBP (aa 1641–2063) as bait. In a temperature-sensitive yeast strain (Cdc 25), protein-protein interactions activate the Ras pathway resulting in yeast survival at the restrictive temperature. Thus, protein interactions were detected by yeast proliferation on galactose plates at 36°C. A glucose plate at 24°C was used as a control to indicate the presence of each clone.

**Comparison of gene and cDNA sequences and noting the presence of GT-AG consensus sequence.**

**Recombinant Protein Binding Assays—** The GST and GST fusion proteins (TRBP-C, CoAA, and CoAM) were produced in Escherichia coli BL21 (DE3) and purified by glutathione-Sepharose resin (Amersham Pharmacia Biotech). In vitro binding assays were performed by incubating GST resin (20 μl, 2 μg) and [32P]Smethionine-labeled, in vitro translated proteins (5 μl) produced by rabbit reticulocyte lysate (Promega). Proteins were incubated at room temperature for 1 h in the binding buffer (20 mM HEPES, pH 7.4, 50 mM NaCl, 75 mM KCl, 1 mM EDTA, 0.05% Triton X-100, 10% glycerol, 1 mM dithiothreitol). Bound proteins were washed 3 times with binding buffer and subjected to SDS-PAGE and autoradiography. For Ku70 and PARP interaction studies, GST resin was incubated with 200 μl of GH3 nuclear extract at 4°C for 16 h in the binding buffer as above with additional 10 μg leupeptin, 10 μg/ml aprotinin, and 10 μg/ml trypsin inhibitor. Bound proteins were detected by Western blotting (ECL). Anti-Ku86 monoclonal antibodies were obtained from NeoMarkers and anti-PARP monoclonal antibody from Transduction Laboratories.

**Northern Analysis—** Human multiple tissue and human cancer cell line Northern blots were obtained from CLONTECH. The CoAA probe was prepared with random-primed 32P-labeled CoAA cDNA (aa 307–584). Northern analysis was performed according to the manufacturer’s protocol.

**Cell Culture and Transient Transfection—** CV-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 0.1 μg/ml penicillin/streptomycin in 5% CO2 at 37°C. Cells were plated in 24-well plates 2 days before transfection. CV-1 cells were transfected using LipofectAMINE reagent according to the manufacturer’s protocol (Life Technologies, Inc.). Cells were incubated with fresh medium containing the indicated concentrations of ligands 16–24 h after transfection. After 24 h, cells were harvested, and luciferase activities were determined as described (8). Total amounts of DNA for each well were equilibrated by adding vector pCDNA3 (Invitrogen). Data are shown as means of triplicate transfections ± S.E.

**CoAA interacts with TRBP in yeast.** CoAA was tested with Sos/TRBP (aa 1641–2063) as bait in the Sos-Ras yeast two-hybrid system. Fox and SoxJun DNA binding domain and empty vectors were used as positive and negative controls, respectively. Protein interaction was detected by yeast proliferation on galactose plates at 36°C. A glucose plate at 24°C was used as a control to indicate the presence of each clone.
employing 5'- and 3'-RACE with mRNA from HeLa cells. Further RT-PCR analysis revealed an additional shorter splice variant. Based on their functional characteristics described below, we designated the full-length clone as coactivator activator, CoAA, and the short splice variant as coactivator modulator, CoAM.

**FIG. 2.** Nucleotide and amino acid sequences of human CoAA and CoAM. CoAA/CoAM cDNA nucleotide and deduced amino acid sequences are shown. Numbers on the left indicate nucleotides, and numbers in parentheses on the right indicate amino acids. The asterisks indicate the stop codons. The TRBP-interacting domain homologous to the rat sequence obtained from the library screen is indicated by the box. The two RRMs of CoAA/CoAM are underlined. CoAM is related to CoAA by alternative splicing, and the splice donor and acceptor sequences at 544 and 1896 positions (gt-ag) are highlighted. The splicing event results in a frameshift resulting in CoAM with a predicted protein sequence containing 156 amino acids as shown in bold. Multiple XYYXXQ sequences (where X is a small amino acid residue including G, A, S and P) primarily located in the TRBP-interacting domain are indicated with tyrosines (Y) circled.
The CoAA cDNA contains 2781 nucleotides and encodes a protein of 669 amino acids with an estimated molecular mass of 69 kDa (Fig. 2). CoAA belongs to the family of RNA-binding proteins because of the presence of two well-characterized RNA recognition motifs (RRMs) within its N terminus (aa 3–68 and 81–144) (Figs. 2 and 3A). Each of the RRM s is composed of two conserved RNP-1 and RNP-2 consensus motifs (Fig. 3B). The crystal structure of RRM has suggested (28, 29) that the RRM motif consists of four anti-parallel ß-strands and two ß-helices that collectively serve as an RNA binding platform. In addition, CoAA also contains a TRBP-interacting domain (aa 307–584) outside the RNA recognition motif (Figs. 2 and 3A). The primary sequence of the TRBP-interacting domain of human primary sequences of these regions have 98% identity. However, evidence suggests that the interspersed aromatic residue repeats among the small residues are important for protein-protein interactions between hnRNP proteins including G, A, S, and P) is present with 20 copies throughout the domain. The function of this motif remains to be further elucidated. However, evidence suggests that the interspersed aromatic residue repeats among the small residues are important for protein-protein interactions between hnRNP proteins (33, 34). This also implies that the entire TRBP-interacting region may be a single functional domain. Thus, the XYXXQ motif might be important for the interaction between TRBP and CoAA.

The alternative splice form, CoAM, was repeatedly and consistently detected by RT-PCR from various tissues and cells (see below). Comparison of CoAA and CoAM sequences indicates the absence of nucleotides 544–1897 in CoAM relative to CoAA cDNA and suggests an alternative splicing of the primary transcript (Fig. 2). The splicing event results in the absence of the TRBP-interacting domain in CoAM (Fig. 2 and 3A). The boundaries of the spliced nucleotide sequence present in CoAA were flanked by consensus splice acceptor and donor GT-AG sequences. This splicing event generates a frameshift at amino acid 150 with the subsequent early termination of translation (Fig. 2). The deduced protein sequence of CoAM contains 156 amino acids, with a calculated molecular mass of 17 kDa. It mostly contains the two RNA recognition motifs that are shared with CoAA.

**Genomic Structure of the Human CoAA/CoAM Gene**—The cDNA sequences obtained by 5′- and 3′-RACE were used to identify the human CoAA/CoAM genomic sequences from the GenBank™ htg data base. The human CoAA/CoAM gene is located on chromosome 11q13 and contains three exons spanning ~11 kilobase pairs (Fig. 3C). Alternative splicing of exon 2 (cDNA nucleotides 544–1897) results in the lack of the TRBP-interacting domain and the formation of CoAM-encoding mRNA. Hence, CoAM is largely composed of two RRM domains.

**CoAM mRNA Is a Natural Transcript**—Several lines of evidence support the conclusion that CoAM is a naturally occurring splice variant and not a PCR artifact. First, several different primer sets yielded nine CoAM RT-PCR products derived from HeLa cell mRNA that correctly corresponded to their predicted sizes, suggesting the RT-PCR results of CoAM were not accidental (Fig. 4, A and B). Second, comparison of the sequences derived from 3′- and 5′-RACE of CoAA with the RT-PCR results for CoAM reveals that the splice site is flanked by splice donor and acceptor consensus GT-AG at nucleotides 544–1897 of the CoAA cDNA sequence (Fig. 2). Third, alternative splicing at nucleotide 1897 corresponds precisely to the splice site at the boundary of the intron and exon 3 when genomic sequence was compared, consistent with alternative splicing at nucleotide 1897 as a naturally occurring event (Fig. 3C). Fourth, the same CoAM sequence was obtained from different sources of human tissues including ovary and liver (Fig.
4C). Finally, a primer crossing the junction of the splice site (B5), which only hybridizes with CoAM but not CoAA, was able to detect the CoAM sequence by RT-PCR (Fig. 4C). All together, these data demonstrate that CoAM is a naturally existing transcript.

**CoAA Interacts with the General Coactivators TRBP and CBP/p300 in Vitro**—In vitro GST pull-down analyses were performed with recombinant GST fusion proteins and [35S]methionine-labeled in vitro translated proteins. Six GST-TRBP fragments were generated in order to map the region(s) required for CoAA interaction (Fig. 5A). The TRBP C terminus (aa 1242–2063 and 1641–2063) and the LXXLL-containing region (aa 719–999), but not GST alone or other regions of TRBP, interacts with CoAA (Fig. 5B). Although the interaction between CoAA and the TRBP LXXLL-containing region was unexpected, CoAA did strongly interact with the TRBP C-terminal regions present in both TRBP-5 and TRBP-6. In addition, this interaction was CoAA-specific since CoAM did not interact with TRBP under the same conditions (Fig. 5B). The protein domain(s) involved in the interaction with CoAA is currently unknown. Thus, it is unclear whether multiple CoAA-interacting domains might be present in TRBP. However, the data were consistent with the yeast assay showing that the TRBP C terminus is capable of interacting with CoAA (Fig. 1). Similar positive interaction results were also obtained when GST-CoAM (Fig. 5C, right panel) was used together with in vitro translated TRBP-6. Luciferase served as a negative control, which did not bind to either. Furthermore, CoAA was able to interact with p300 (Fig. 5D). The interaction of CoAA with p300 requires the C-terminal region of p300. These results suggested that CoAA directly interacts with coactivator TRBP and CBP/p300 in vitro. CoAM, which contains the two RNA recognition motifs but not the TRBP-interacting domain, was not able to bind to TRBP. This indicates that the major C-terminal region of CoAA, which includes the TRBP-interacting domain and is composed of multiple YXXQ motifs, is largely responsible for the interaction between TRBP and CoAA.

**Ubiquitous Expression of CoAA**—We examined the expression pattern of endogenous CoAA mRNA using human Northern blots containing different tissues and cell lines. The blots were probed with a cDNA encoding the CoAA-specific TRBP-interacting domain. A predominant 3.0-kilobase pair transcript was detected, which was present in all the human tissues tested including brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood lymphocytes (Fig. 6). CoAA was also present at comparable levels in HL-60, HeLa, K-562, MOLT-4, Raji, SW480, A549, and G-361 human cancer cells (Fig. 6). As general coactivators such as TRBP and CBP/p300 are ubiquitously expressed, the wide distribution of CoAA suggests a potential function of CoAA in modulation of the general transcriptional machinery.

**Fig. 4.** CoAM is a natural transcript present in different human tissues. A, schematic representation of CoAA and CoAM with depiction of the primers for RT-PCR. Primer B5 encompasses the splice site junction that will bind CoAM selectively. B, RT-PCR analysis of CoAM using HeLa mRNA and primer sets shown in A. Sequences of the primers are described under “Experimental Procedures.” C, PCR analysis of CoAA and CoAM using first strand cDNAs from human ovarian and liver tissues. CoAM cDNA and HeLa first strand cDNA were used as positive controls. CoAA cDNA was used as a negative control for the CoAM selectivity of primer B5. Primer sets are shown in A. M denotes a 1-kilobase pair ladder of nucleotide markers.

**Fig. 5.** CoAA interacts with TRBP and p300 in vitro. GST fusion proteins (TRBP, CoAA, and CoAM) were incubated with in vitro translated, [35S]methionine-labeled CoAA, CoAM, TRBP, or p300. Bound proteins or fragments were resolved by SDS-PAGE and detected by autoradiography. CoAA and CoAM were full-length, and TRBP and p300 were fragments as indicated. GST alone and luciferase were used as negative controls. A, schematic diagram illustrating the regions of TRBP used for mapping of the CoAA-interacting domain. Numbers indicate positions of amino acids. B, [35S]methionine-labeled CoAA and CoAM were tested with GST-TRBP fragments. Input is 10%. C, GST-CoAA and GST-CoAM were tested with [35S]methionine-labeled TRBP-8 (aa 1641–2063). Luciferase was used as a negative control. D, GST-CoAA was tested with [35S]methionine-labeled p300 fragments, as indicated. GST alone served as a negative control.
CoAA Acts as a Potent Coactivator for Nuclear Receptors—Given that CoAA interacts with the nuclear receptor coactivator TRBP, we investigated the role of CoAA in nuclear receptor-mediated transcriptional regulation. The mouse mammary tumor virus promoter (MMTV) luciferase reporter was tested in CV-1 cells with the glucocorticoid receptor (GR) in the presence or absence of dexamethasone (Dex). CoAA transactivated the MMTV promoter 10-fold greater than the vector control (Fig. 7A). This activation was Dex-inducible and GR dose-dependent, suggesting that CoAA action is mediated by GR (Fig. 7, A and B). The activation by CoAA was also CoAA dose-dependent (Fig. 7C). The potency of CoAA activation was similar when compared with coactivators TRBP and CBP, and the actions of these three coactivators were strongly synergized when transfection conditions were optimized (Fig. 7D). Consistent with the previous binding data, these results suggest that CoAA is a potent nuclear receptor coactivator and might enhance transcription through other coactivators such as TRBP and CBP/p300.

TRBP-interacting Domain Is Required for CoAA Activation—In order to map the domains required for CoAA transactivation function, we generated CoAA deletion mutants in which RRMs and TRBP-interacting region were selectively deleted. CoAA and CoAM were compared with several CoAA deletion mutants in a transient transfection assay. As shown in Fig. 8, while CoAA stimulated Dex-regulated transcription, CoAM strongly repressed transcription to a level below that of the vector control. Deletion of the two RRM domains (CoAA-1) completely abolished the CoAA activity indicating that the RRMs are required for CoAA activation. The TRBP-interacting domain itself (CoAA-2) was not active, suggesting that the loss of function was due to the absence of RRMs, rather than the presence of an inhibitory element close to the TRBP-interacting region. Deletion of the TRBP-interacting region reduced but did not abolish the CoAA activity (CoAA-3), whereas further deletion to remove an additional XXXQ-containing region (CoAA-4) almost fully abolished the activity of CoAA. From a protein structural point of view, and the sequence of CoAA (Fig. 9A), we hypothesized that the XXXQ-containing region (~201–584 aa) might be a single domain that is responsible for the coactivator interaction. Indeed, the TRBP-interacting domain is likely larger than the region obtained from the yeast two-hybrid screen (aa 307–584). Deletion of the entire domain (aa 201–584) (CoAA-4) impaired the function of CoAA, suggesting that the TRBP-interacting domain (aa 201–584) is important for CoAA transactivation in addition to the RRMs. Taken together, the above data suggest that both RRMs and the TRBP-interacting domain are required for full function of CoAA.

CoAM Modulates the Transcriptional Activities of Coactivators Including CoAA, TRBP, and CBP—Strikingly, lacking a TRBP-interacting domain, CoAM dramatically repressed transcription in a similar transient transfection system (Figs. 7A, 8, and 9). In Fig. 9A, CoAM shows a dose-dependent repression of transcription when compared with the vector control. These data indicate that CoAM might repress the function of endogenous coactivators. When CoAA was cotransfected with increasing amounts of CoAM, CoAA also had a dose-dependent repression of CoAA-stimulated transactivation (Fig. 9B), suggesting that CoAM may compete with CoAA with regard to RR function. Furthermore, in cotransfection studies where CoAA, TRBP, CBP, and CoAM were coexpressed, CoAM significantly repressed TRBP or CBP activity to or below the basal level (Fig. 9C). This indicates that TRBP and CBP may require other activities, possibly including the endogenous CoAA, in order to function as coactivators. CoAA activity was regulated by CoAM, which shares two RRMs with CoAA. CoAA was repressed by CoAM to a lesser extent when compared with TRBP and CBP (Fig. 9C), presumably because overexpression of CoAA overcomes the competition with CoAA to a greater
NF-κB luciferase reporters containing GRE, TRE, ERE, AP-1, and possibility, CoAA and TRBP were tested with a number of activate multiple hormone response elements. To examine this its interaction with TRBP, might possess a similar ability to type CoAA and deletion mutants.

The schematic diagram below the graph illustrates the various wild-type CoAA and deletion mutants. Numbers indicate positions of amino acids in CoAA. Cells were grown in the presence or absence of 100 nM dexamethasone (DEX).

extent than for TRBP and CBP.

CoAA Functions as a General Coactivator on Multiple Hormone Response Elements—The general coactivator TRBP activates nuclear receptors as well as other transcription factors such as AP-1 and NFκB (8). We speculated that CoAA, through its interaction with TRBP, might possess a similar ability to activate multiple hormone response elements. To examine this possibility, CoAA and TRBP were tested with a number of luciferase reporters containing GRE, TRE, ERE, AP-1, and NFκB enhancer binding sites. The results indicate that CoAA and TRBP are synergistic on all of these elements (Fig. 10). Thus, CoAA also functions as a general coactivator for transcription mediated by nuclear receptors and other transcription factors.

CoAA Interacts with the DNA-PK/PARP Complex—One of the interesting features of coactivator TRBP is that its C-terminal region associates with the DNA-PK/PARP complex and can be phosphorylated by DNA-PK (8). Accumulating evidence suggests that nuclear complexes involved in transcription, DNA recombination, and DNA repair are colocalized in vivo and share components such as DNA-PK and PARP (30). Since CoAA is an active component associated with TRBP, we examined whether CoAA might be one of the factors in this complex. Pull-down assays using GH3 nuclear extract show that CoAA interacts with the DNA-PK regulatory subunit Ku86 and PARP (Fig. 11), although the interactions may be either direct or indirect in vivo. These results are consistent with CoAA playing an important role in the regulation of general transcription.

DISCUSSION

The Sos-Ras two-hybrid system detects protein-protein interactions in yeast cytoplasm rather than the nucleus (25–27). This may confer an advantage over the conventional yeast two-hybrid system when identifying proteins with transactivation domains. This system has significantly facilitated the search for novel coactivators. We have successfully obtained several coactivators including TRBP using this system (8).

TRBP is notable for its large size, high potency in transient transfections, unique structural features, and low in vivo abundance. Like CBP, TRBP appears to be promiscuously active on multiple response elements. We also have demonstrated that TRBP associates with nuclear components including CBP/p300, suggesting its close linkage with the common transcriptional apparatus and its critical role in nuclear function. Hence, as a TRBP-interacting coactivator, CoAA may also play an important role in transcription.

It is noteworthy that the coactivator CoAA belongs to a ribonucleoprotein family. Ribonucleoproteins are RNA-binding proteins that have long been known to be important for nuclear function (21, 22). In fact, together with nuclear RNAs, they are major contributors to nuclear structure. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of RNA-binding proteins that were originally named based on their heterogeneous size. hnRNPs bind to hnRNAs and pre-mRNA derived from nascent pol II transcripts and are involved in mRNA processing and turnover. Most biochemically identified hnRNPs are abundant proteins that interact with RNA and serve as "RNA histones" and shuttle between the nucleus and cytoplasm. Some hnRNPs have also been shown to play roles, although less defined, in transcription, DNA replication, and recombination (17).

Historically, hnRNPs are purified as a nuclear complex and identified using two-dimensional gels. Inasmuch as most coac- tivators are not sufficiently abundant to detect in this manner, CoAA may represent a new class of low abundance hnRNPs that were not identified previously. Recent studies suggest that

![Figure 8](https://example.com/figure8.png)  
**FIG. 8.** TRBP-interacting domain is required for the transcriptional activity of CoAA. CV-1 cells were cotransfected with MMTV luciferase reporter (100 ng), GR (10 ng), control vector pcDNA3 (V, 200 ng), or wild-type CoAA, CoAM, or CoAA deletion mutants (CoAA1–4). The schematic diagram below the graph illustrates the various wild-type CoAA and deletion mutants. Numbers indicate positions of amino acids in CoAA. Cells were grown in the presence or absence of 100 nM dexamethasone (DEX).

![Figure 9](https://example.com/figure9.png)  
**FIG. 9.** CoAM modulates coactivator-mediated transcription. CV-1 cells were cotransfected with MMTV luciferase reporter (100 ng), GR (10 ng), and additional plasmids as described below. Cells were grown in the presence or absence of 100 nM dexamethasone (DEX). A, CoAM inhibits endogenous level of transcription. Cells were cotransfected with increasing amounts of CoAM (0, 10, 20, 50, 100, 200, and 400 ng) as indicated. B, CoAM inhibits CoAA-mediated transactivation. Cells were cotransfected with increasing amounts of CoAM (0, 75, 150, and 300 ng) and a constant amount of CoAA (150 ng). C, inhibition of activities of coactivators TRBP and CBP by CoAM. Cells were cotransfected with 150 ng of CoAA, CoAM, TRBP, or CBP as indicated.
hnRNPs, in addition to RRMs, is a Gly-rich domain with interspersed aromatic residue repeats, which has been shown to be responsible for protein-protein interactions among hnRNPs (33, 34). CoAA has two RRMs and a TRBP-interacting domain that together structurally resemble key features of hnRNPs. The TRBP-interacting domain of CoAA contains a significant number of repeats of tyrosines nested among the small amino acid residues such as alanine, serine, and glycine. This feature may serve as a coactivator-binding motif, analogous to Gly-rich domains in hnRNPs.

The other well known feature of hnRNPs is the existence of splice variants. CoAM mRNA is an alternative splice form of the CoAA transcript and encodes a protein with two RRMs identical to those present in CoAA but without the TRBP-interacting domain. We have provided several lines of evidence suggesting that CoAM mRNA is a result of a natural splicing event. Although the relative amounts of endogenous CoAA and CoAM proteins in tissues are not known, the balance of CoAA and CoAM in vivo could be physiologically relevant and determine transcriptional activities of target genes in each tissue. Transient transfection studies showed that CoAA stimulates, and CoAM represses, TRBP-mediated transcription (Fig. 9). These data not only suggest that CoAA interaction with TRBP is important for CoAA activation but also indicate that putative RNA binding through its RRMs of CoAA may be important for transcriptional activation. In this instance, CoAA completely prevented TRBP and CBP/p300-mediated transcriptional activation, presumably blocking CoAA action (Fig. 9C). Inasmuch as CoAA effects were not promoter-specific, the involvement of DNA via coactivators could be a common mechanism underlining an aspect of transcription regulation. On the other hand, CoAA appears not to contain the LXXLL motif or a binding domain for other transcription factors, suggesting CoAA might represent another class of coactivators that are functionally distinguishable from TRBP and CBP/p300.

Collectively, the above data suggest that CoAA might exert its transactivation activity through its putative interaction with an RNA-containing complex via the RRMs domains and interaction with coactivators via the TRBP-interacting domain. On the other hand, CoAM might compete with endogenous CoAA and other putative RRM-containing endogenous coactivators for the binding of an RNA-containing transcriptional complex. This, in turn, may inhibit transcriptional activation. The general coactivators TRBP and CBP may require CoAA to interact with the transcriptional machinery. In this case, blocking the interaction sites would prevent coactivator access to the transcriptional complex. Taken together, CoAM apparently interacts with nucleic acids. The interaction of RRM with its cognate RNA hairpin loop is hypothesized to occur via an induced fit mechanism (28). Another structural feature of many hnRNPs, in addition to RRMs, is a Gly-rich domain with interspersed aromatic residue repeats, which has been shown to be responsible for protein-protein interactions among hnRNPs (33, 34). CoAA has two RRMs and a TRBP-interacting domain that together structurally resemble key features of hnRNPs. The TRBP-interacting domain of CoAA contains a significant number of repeats of tyrosines nested among the small amino acid residues such as alanine, serine, and glycine. This feature may serve as a coactivator-binding motif, analogous to Gly-rich domains in hnRNPs.

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acts as a dominant negative regulator for CoAA and may modulate CoAA function in vivo.

The machinery for transcription, DNA replication, and recombination have been proposed to be colocalized with functional overlap (18). The association of CoAA with DNA-PK and PARP complexes further suggests that CoAA might be localized within the same nuclear machinery. Like most other biological phenomena, transcriptional regulation is likely a sequential process that involves multiple components and steps. In this view, CoAA may be an important player in this event.

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