Cell type specific regulation of COUP-TF II promoter activity.

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Abstract COUP-TF family orphan receptors regulate activity of ligand-activated nuclear hormone receptors or function independently in the regulation of gene expression. COUP-TF II has a complex expression pattern suggesting that different mechanisms are involved in the regulation of its expression. We isolated the 5′ regulatory region of the mouse COUP-TF II gene and demonstrated that the basal promoter is localized in a −200 bp region 5′ from the transcription start site. All-*trans* retinoic acid and dibutyryl cyclic AMP have cell type specific effects on COUP-TF II promoter activity. The effect of cyclic AMP is mediated by the cyclic AMP response element that is localized 74 nucleotides upstream from the major transcriptional start. In vitro promoter analyses also demonstrated that the effect of all-*trans* RA is not directly mediated by the binding of RARs or RXRs to the promoter sequence.

Key words: COUP-TF II; Gene regulation; Promoter; Retinoic acid; Dibutyryl cyclic AMP

1. Introduction

Nuclear hormone receptors play an important role during development of different cell types and organ systems [1,2]. Retinoic acid (RA) receptors, retinoid X receptors, thyroid hormone receptors, steroid hormone receptors, and several other members of the superfamily of nuclear hormone receptors regulate expression of a variety of target genes [3,4]. These ligand dependent transcription factors bind as homo- or heterodimers to hormone response elements (HRE) located in the regulatory regions of target genes and regulate transcription [3]. Recently, it has been shown that nuclear hormone receptors can also suppress transcriptional activity by complexing with non DNA binding co-repressors [5–8]. Beside the ligand activated transcription factors, the nuclear hormone receptor superfamily comprises orphan receptors for which ligands are not known [9,10]. Orphan receptors, such as chicken ovalbumin upstream promoter transcription factor (COUP-TF) homologs, have been isolated from several species including rodents and human [11–21]. COUP-TF orphan receptors function as transcriptional activators or suppressors depending on the complexes they form and DNA sequences on which they bind. One function of the COUP-TFs is to regulate the activity of ligand activated nuclear hormone receptors [22,23]. Several different mechanisms may contribute to the repression of induction including direct competition of COUP-TFs for the hormone response elements, heterodimerization with retinoid X receptors (RXR) and suppression of transcription by COUP-TF homodimers [22,24–27]. Repression by COUP-TFs may also sensitize promoters of different genes to positively acting nuclear hormone receptors [26]. COUP-TFs may also function as a stimulatory transcription factors. COUP-TFs stimulate transcription of arrestin gene by binding to a direct repeat with a 7 bp spacer located upstream of the transcription start site [28]. In combination with HNF-4 transcription factor, COUP-TF I and COUP-TF II may also stimulate transcription of the phosphoenolpyruvate carboxykinase gene [29].

Complex expression patterns of COUP-TF I (Ear-3) and COUP-TF II (ARP-1) during development argue for their functional roles in several developmental processes. While the potential functions of COUP-TFs have been extensively investigated, the molecular mechanisms responsible for the spatial and temporal expression of COUP-TFs are still poorly understood. Expression of COUP-TFs in different regions of developing and adult organisms indicates that different mechanisms are involved in the regulation of their spatial and temporal expression.

Here we demonstrate that COUP-TF II basal promoter is localized in the 200 bp region 5′ from the transcription start site, and that all-*trans* retinoic acid and dibutyryl cyclic AMP have cell type specific effects on COUP-TF II promoter activity. Also, COUP-TF I and COUP-TF II weakly inhibit RA induced COUP-TF II promoter activity in PCC7 cells.

2. Materials and methods

2.1. Plasmid construction

A mouse genomic DNA library (Clontech) was screened with mouse COUP-TF II cDNA probe (isolated in our laboratory) using high stringency conditions (0.1 × SSC, 65°C; 1 × SSC = 150 mM NaCl, 15 mM Na-citrate). Fragments of isolated clones were subcloned into Bluescript II KS plasmid (Stratagene) for sequencing and generation of promoter constructs. All the COUP-TF II promoter fragments were cloned into the unique *Bgl*II site of the vector pCAT3N using *Bgl*II linkers. COUP-TF II promoter fragments of various lengths were generated by digestion of 5′ regulatory region DNA with endonucleases listed below: for plasmid −4000/*Bgl*II with *Bam*HI and *Bgl*II, for −1500/*Bgl*II with *Hin*dIII and *Bgl*II, for −621/*Bgl*II with *Bst*XI and *Bgl*II, for −320/*Apa*I with *Apa*I, for −97/*Apa*I with *Sty*I and *Apa*I, for −320/*Sac*II with *Apa*I and *Sac*II, for −40/*Sac*II with *Sac*I and *Sac*II, for −40/*Apa*I with *Sac*I and *Apa*I, and for *Sac*II/*Apa*I with *Sac*II and *Apa*I. Constructs −258/*Apa*I and −200/*Apa*I were generated by digestion of *Apa*I fragment with Exonuclease III. Construct Δ*Sty*I/*Apa*I was generated by deletion of *Sty*I fragment from the −320/*Apa*I plasmid.

Site directed mutagenesis was performed using plasmid −320/*Apa*I and oligonucleotides 5′-GTTGCAGCAGTCGTGATGCATTTCACTATATAGAGAG (mut 1) and 5′-ACGTGCGCTAAGTTGCATATGTCGTGTCAAAGTTCACT (mut 3). Erase-a-Base System (Promega) was used to generate mutations mut 2 and mut 4 by digesting plasmids mut 1 and mut 3 with *Nsi*I and *Nde*I, respectively.

Mouse COUP-TF I and COUP-TF II (cloned in our laboratory) cDNAs were cloned into eukaryotic expression vector pRcCMV-neo (Invitrogen) using appropriate restriction endonucleases.

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A

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BstXI
TGGTTTGCAAGCCCAGATCCCGGCACCTTGCCACCTCCGCTGCGTACCCCTCTACAAAGA -562
AGGGGAGAGCATTATTCAGTCTTTTGGAATAGTGGGTTCTACATAATGCGCCGGGAGTCC -502
CGGTGGACCGCGAGCTCGCATTTAGAGGCATCGCAAGTTGCGAGAGACTAAGTCTTTGGC -442
TCCATCCTCAACCCCTTGCGGACGCTTTAAGAGTCGTAGTGTGTGCGCCGCACTAGTCTT -382
GCGCAGGGCGGAAACCACTGTGCCGATGCGGCGGCGGGGTCCGCGCTCGGCTGCCACCGG -322
ApaI
GCCCCCATCCCCCTCTCTGTGGCCAGGACCGCGCGGCCACCCGTCCCCGCCCCCCTCCGT -262
CCCCGCGCGCCCCGTGCGCTTGTCTCGACGCCGCTCGCGCTAGGACCGGGCTGCTCCCGC -202
      StyI
TGCCGCCATGGCGGGCCGCTGCGCTCCGGCCAATGACGACGAGGGGGCGGCGCGCGCGCG -142
                                                  StyI
CGGCCGGGCCAACCCCGGCGGCTGCCTTATAAGGCGGCCCGTCGCCATGGCAACGTGCGC  -82
                          * *          SacI        * *
TAAGTTGCAGCAGTCGTGTCAAAGTTCACTATATAGAGAGCTCAGTGAGTGATCGAGGAG  -22
                       * *1*                       * *
AAGCAACTTCTGCCAGCCCGGAGCCTATAAATCGCATTCCCTCCCGAGCCCCCCTTTTTA   37
                                    SacII
GCATATTTGATCACTTTGATTCTGCTCTTTTCTCTCCGCGGTGTGTGCGTCGGTGCGCGC   97
GTGTGTGTTTTCTTCTCCTCCTCCTCCTCTGCGAGTTGCCTCCTTTCTCCGGGTGCGGCT  157
GCTCTTCCCTCTTTCATTCTTTCTCTCCGTTTCTCCCCCCTCTGCGCACGAAGGATGCGC  217
TTCTAGGTGCTGATCTGCCCTCCTTTCTCTCTTATCATCTCTCCCGCCGCCGGCGAGTTG  277
ACTCTTTCCCTATTGCTGCTTGAGGTGTGCGGCAGCAGCAGCAGCAGCAGCAGCAGC    337
GGCTCCACCGGCGGCGGCAGCAGCGGCAGCAGCAGCAGCGACTCCACCGGGGACGGCGGG  397
                  ApaI
TAGACGCAGCGGCTCCGGGCCC
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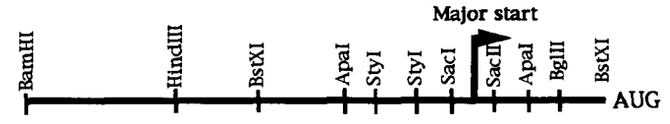
B

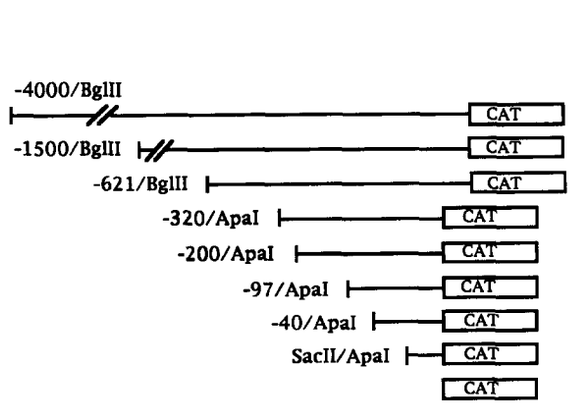


	CAT ACTIVITY % conversion		
	PCC7	3T3	C33A
-4000/BglII CAT	8 ± 1	9 ± 2	54 ± 4
-1500/BglII CAT	10 ± 2	9 ± 1	67 ± 6
-621/BglII CAT	12 ± 2	11 ± 2	75 ± 6
-320/ApaI CAT	12 ± 2	10 ± 2	73 ± 5
-258/ApaI CAT	11 ± 2	11 ± 1	73 ± 6
-200/ApaI CAT	12 ± 2	11 ± 2	74 ± 6
ΔStyI/ApaI CAT	5 ±0.6	6 ±0.4	24 ± 2
-97/ApaI CAT	3 ±0.4	2 ±0.2	13 ± 1
-320/SacII CAT	11 ± 2	13 ± 2	74 ± 5
-40/SacII CAT	0.4±0.04	0.3±0.02	0.8±0.03
-40/ApaI CAT	0.5±0.03	0.4±0.02	0.8±0.03
SacII/ApaI CAT	0.1±0.03	0.1±0.05	0.2±0.06
CAT	0.1±0.05	0.2±0.04	0.1±0.06

Fig. 1. Nucleotide sequence of the mouse COUP-TF II 5′ regulatory region (A) and its promoter activity in mouse embryo carcinoma PCC7, mouse fibroblast 3T3, and human cervical carcinoma C33A cells (B). (A) Nucleotide numbering starts at the major transcriptional start site (1), upstream nucleotides have negative numbers. Minor start sites are indicated by * (above the corresponding nucleotides). The TATA-box core motifs are indicated by boldface letters, and restriction sites used in generation of CAT constructs are indicated. (B) In the schematic representation of mCOUP-TF II promoter region restriction sites and major start site (arrow) are indicated. The data shown are representative of at least three independent CAT assays.

2.2. Cell culture

Embryonic carcinoma cell line PCC7 was obtained from S. Pfeiffer and grown in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Summit Biotechnology). PCC7 cells were



	CAT ACTIVITY % conversion			
	CONTROL	RA	dBcAMP	dBcAMP+RA
-4000/BglII	2.5 ± 0.5	11 ± 2	10 ± 1	38 ± 3
-1500/BglII	2.8 ± 0.3	9 ± 1	11 ± 1	41 ± 3
-621/BglII	2.3 ± 0.2	11 ± 2	10 ± 1	39 ± 2
-320/ApaI	2.0 ± 0.1	10 ± 1	9 ± 1	42 ± 2
-200/ApaI	2.1 ± 0.3	11 ± 1	10 ± 1	42 ± 3
-97/ApaI	0.4 ± 0.05	3 ± 0.3	4 ± 0.5	9 ± 1
-40/ApaI	0.3 ± 0.05	0.3 ± 0.04	0.3 ± 0.05	0.4 ± 0.06
SacII/ApaI	0.2 ± 0.06	0.1 ± 0.05	0.2 ± 0.06	0.1 ± 0.05
CAT	0.1 ± 0.06	0.2 ± 0.05	0.2 ± 0.05	0.1 ± 0.06

Fig. 2. Effects of all-*trans* retinoic acid and dibutyryl cAMP on COUP-TF II promoter activity in CAT assay using mouse embryo carcinoma PCC7 cells. Cells were treated with all-*trans* RA (0.5 μM), dBcAMP (1 mM) or combination of all-*trans* RA and dBcAMP. The data shown are representative of at least three independent experiments.

differentiated into neuronal-like cells with all-*trans* RA (0.5 μM) and dibutyryl cyclic AMP (dBcAMP; 1 mM) treatment. Mouse fibroblast 3T3, rat glioblastoma C6, human glioblastoma U373, and human cervical carcinoma C33A cells were obtained from the American Type Culture Collection and were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

2.3. DNA transfection and CAT assays

Cells were transfected with 15 μg of total plasmid DNA, using the calcium phosphate precipitation method [30] in 60 mm diameter dishes (1×10^5 to 3×10^5 cells per dish). The medium was changed 12–18 h after transfection to normal growth medium or medium containing all-*trans* RA (5×10^{-7} M) or dBcAMP (1 mM) as indicated for each experiment. Cells were harvested 48 h later. Cells were washed and harvested in PBS, lysed in 150 μl of 0.25 M Tris-HCl (pH 7.6) by freeze/thawing three times, and incubated at 65°C for 10 min to minimize deacylation activity. Protein concentration in the lysates was determined by a protein assay reagent (Pierce Chemical Co.) with BSA used as a standard. A 150 μl mixture containing 0.4 mM acetyl coenzyme A, 0.1 μCi of [dichloroacetyl-1,2-^{14}C]chloramphenicol, and 10–25 μg of protein was incubated at 37°C for 0.5–1.5 h. After extraction with ethyl acetate, the radioactive forms of chloramphenicol were resolved by thin-layer chromatography. Quantitation of acetylation ratios was obtained by PhosphorImager (Molecular Dynamics) analysis. To normalize transfection efficiencies, cells were cotransfected with 1 μg of plasmid pRcRSVlacZ. All the reported CAT activities were normalized to total protein and lacZ activity. The CAT assay values represent the means of at least three independent transfections.

3. Results

3.1. Analysis of COUP-TF II gene promoter region

We isolated a phage clone containing the 5′ end of the COUP-TF II gene and sequenced a 1.5 kb fragment that extended for 622 nucleotides upstream of the major transcription initiation site (Fig. 1A). The transcription initiation sites were mapped by primer extension and RNase protection analyses (data not shown) using RNA isolated from embryonic day 11, 13, and 15 mouse embryos. Both methods demonstrated the presence of several transcription start sites (Fig. 1). The sequence of the 5′-proximal region contains several consensus TATA box sequences in the region where all the transcription start sites are localized (Fig. 1A). TATA box sequences in COUP-TF II promoter do not lie at typical distances (20–30 nucleotides) from start sites which is not an unusual situation.

To determine the sequences that are essential for transcription of mouse COUP-TF II gene, various portions of the 5′-flanking region were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene as a heterologous reporter gene. Reporter plasmids containing different fragments of the COUP-TF II gene 5′ region (Fig. 1B) were transiently transfected into the mouse teratocarcinoma PCC7, mouse fibroblast 3T3, and human cervical carcinoma C33A cells. CAT assays demonstrated that COUP-TF II promoter CAT plasmids −4000/*Bgl*II, −1500/*Bgl*II, −621/*Bgl*II, −320/*Apa*I, −258/*Apa*I, −200/*Apa*I, and −320/*Sac*II have similar promoter activity in all studied cell lines (Fig. 1B). Deletion of additional 103 nucleotides (construct −97/*Apa*I) resulted in 4–6-fold reduction of promoter activity. Also, deletion of nucleotides −196 to −97 from −320/*Apa*I construct (plasmid Δ*Sty*I/*Apa*I) resulted in 2–3-fold reduction of promoter activity. CAT constructs containing 40 bp promoter sequences (constructs −40/*Sac*II and −40/*Apa*I) and first exon sequences (construct *Sac*II/*Apa*I) showed almost no CAT activity. This deletion analysis demonstrated that the COUP-TF II basal promoter is localized in the 200 bp region 5′ from the transcription start site.

3.2. All-trans retinoic acid and cAMP induce COUP-TF II promoter activity in teratocarcinoma PCC7 cells and suppress it in C6 and U373 glioma cells

Since COUP-TF II is involved in the modulation of retinoic acid responses and may function as a part of the regulatory loop, we analyzed the effect of all-*trans* RA on the activity of its promoter. Initially, we used teratocarcinoma PCC7 cells which have been shown to express all three RAR and RXR genes [31]. Also, RA treatment induces expression of all three RAR genes in these cells ([31] and our unpublished data). CAT assays following transient transfection by COUP-TF II promoter-CAT plasmids containing different fragments of 5′ regulatory region (−4000/*Bgl*II, −1500/*Bgl*II, −621/*Bgl*II, −320/*Apa*I, −200/*Apa*I, −97/*Apa*I, −40/*Apa*I, and *Sac*II/*Apa*I) demonstrated that RA treatment induces promoter activity of all plasmids which contain more than 40 bp of the promoter region (Fig. 2). These CAT assay data suggest that RA response element(s) are localized in the −97 to −40 nucleotide promoter region. Increased levels of cAMP have been shown to potentiate the effect of RA on neuronal differentiation in teratocarcinoma cells (our unpublished data). Based on

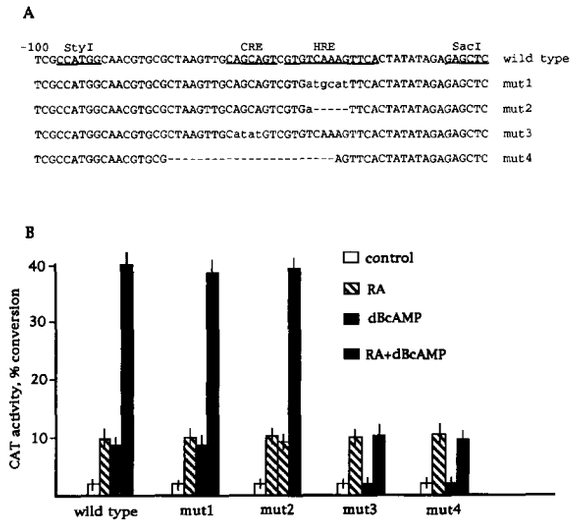


Fig. 3. Effects of mutations in CRE and HRE on mCOUP-TF II promoter activity. (A) Nucleotide sequences of wild type and mutated (mut 1, mut 2, mut 3, mut 4; changed nucleotides are in lower case) promoter regions. (B) Effect of all-*trans* retinoic acid and dibutyryl cAMP on wild type and mutated COUP-TF II promoter activity in transient CAT assay using mouse embryo carcinoma PCC7 cells. The wild type −320/*Apa*I CAT plasmid and mutated reporter plasmids, mut 1, mut 2, mut 3, and mut 4 were transfected into PCC7 cells and cells were treated with all-*trans* RA (0.5 μM), dBcAMP (1 mM) or combination of all-*trans* RA and dBcAMP. The data shown are representative of at least three independent experiments.

these observations, we also analyzed the effect of dibutyryl cAMP (dBcAMP) on COUP-TF II promoter activity in teratocarcinoma PCC7 cells. The same promoter CAT constructs that responded to the RA treatment were also responsive to dBcAMP treatment (Fig. 2). This argues that cAMP response element(s) are localized in −97 to −40 bp region of COUP-TF II promoter. Cotreatment of PCC7 cells with RA and dBcAMP stimulated COUP-TF II promoter activity about 15–20-fold.

Sequence analyses of −97 to −40 bp COUP-TF II promoter region revealed a cAMP response element (CRE) and a possible consensus sequence for hormone response element (HRE) localized next to each other (Fig. 3). Mutations were introduced into these elements to assess their functionality (Fig. 3). COUP-TF II 320 bp promoter CAT plasmids (−320/*Apa*I CAT) containing mutated CRE and HRE were transfected into PCC7 cells, and effects of RA and dBcAMP on promoter activity were analyzed. Surprisingly, mutations which destroyed HRE (mut 1 and mut 2) did not affect inducibility of COUP-TF II promoter activity (Fig. 3). However, mutations in CRE (mut 3) or in both CRE and HRE together (mut 4) completely blocked the effect of dBcAMP on the inducibility of the promoter. These results argue that RA has an indirect effect on COUP-TF II promoter activity in PCC7 cells. Also, the additive effect of RA and dBcAMP suggests that RA and dBcAMP have different pathways to stimulate COUP-TF II promoter activity.

To further explore the role of RA on COUP-TF II promoter activity, we performed CAT assays using mouse fibroblast 3T3, rat glioblastoma C6, human glioblastoma U373, and human cervical carcinoma C33A cells. Treatment of 3T3 and C33A cells with RA and dBcAMP did not affect the activity of COUP-TF II promoter constructs −320/*Apa*I and −4000/*Bgl*II (Fig. 4A,B). On the other hand, both RA and dBcAMP suppressed activity of COUP-TF II promoter constructs in C6 and U373 cells.

3.3. *COUP-TF I and COUP-TF II weakly inhibit RA induced COUP-TF II promoter activity in PCC7 cells*

Transcription factors COUP-TF I and COUP-TF II block the stimulatory effect of RA on transcription of several genes [24–27]. Our data demonstrate that RA induces COUP-TF II promoter activity in PCC7 cells. We then asked whether COUP-TF I and COUP-TF II block the stimulatory effect of RA on COUP-TF II promoter activity. We cotransfected the −320/*Apa*I COUP-TF II CAT construct with COUP-TF I and COUP-TF II expression plasmids into PCC7 cells and analyzed CAT activities. As a positive control, we used a plasmid containing an RARβ2 promoter *lac*Z construct (from A. Zimmer, NIH) that has been shown to be inducible with activated RARs and RXRs. CAT assays demonstrated that both COUP-TF I and COUP-TF II slightly suppress RA and dBcAMP induced COUP-TF II promoter activity (Fig. 4C). By contrast, COUP-TF I and COUP-TF II completely inhibit the RA and dBcAMP induced RARβ2 promoter activity (Fig. 4C). This is an additional indication that RA does not directly regulate COUP-TF II promoter activity through activation of RAR/RXR complexes.

4. Discussion

The central issue addressed in this study is the transcriptional regulation of the COUP-TF II gene. COUP-TF II gene is expressed in a complex spatio-temporal pattern during development of several organ systems including nervous system [19–21,32]. This complex expression pattern argues for a different mechanisms of COUP-TF II gene regulation in different cell types. Our data demonstrate that the effects of RA and dBcAMP on the COUP-TF II promoter activity are different or even opposite in different cell types. Also, treatment of mouse embryos with all-*trans* RA results in stimulation of COUP-TF II expression in the spinal cord and suppression of expression in the telencephalon [33]. Additionally, it has been demonstrated that expression of COUP-TF I and COUP-TF II is induced during neuronal differentiation of teratocarcinoma P19 cells [19,32] but not in several other teratocarcinomas (our unpublished data). Our data support the hypothesis that COUP-TF II gene expression is regulated differently in different cell types and that the same stimuli may regulate its expression in opposite directions depending on the cell type.

Analyses of COUP-TF II promoter activity localized the functional cAMP response element 74 nucleotides upstream from the major transcriptional start site. This CRE is responsible for the stimulatory effect of dBcAMP in teratocarcinoma PCC7 cells. The suppressive effect of dBcAMP on COUP-TF II promoter activity in C6 and U373 cells is not mediated by the same CRE since mutation in this sequence or deletion of it does not change the response of COUP-TF II promoter to dBcAMP in these cells. Sequence analyses of COUP-TF II promoter also identified a possible HRE localized 68 nucleotides from the major transcriptional start site. The sequence of this element does not correspond exactly to HRE, but considering the high variability of HRE sequences, it is possible that this sequence functions as a binding site for RARs or RXRs. Also, CAT assays mapped the RA response element to

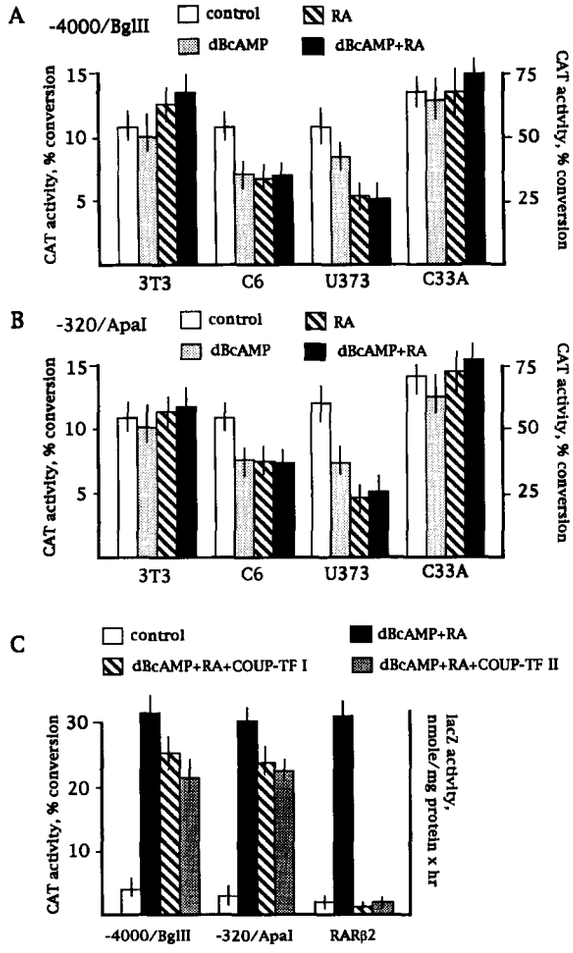


Fig. 4. Effects of all-*trans* retinoic acid, dibutyryl cAMP (A and B), and COUP-TF I and COUP-TF II (C) on COUP-TF II promoter activity in CAT assay using mouse fibroblast (3T3), rat glioblastoma (C6), human glioblastoma (U373), human cervical carcinoma (C33A), and mouse teratocarcinoma (PCC7) cells. (A and B) Mouse COUP-TF II −4000/*Bgl*II (A) and −320/*Apa*I (B) CAT plasmids were transfected into 3T3, C6, U373, and C33A cells. Cells were treated with all-*trans* RA (0.5 μM), dBcAMP (1 mM) or combination of all-*trans* RA and dBcAMP and CAT activities were measured. Left side scale represents CAT activity for 3T3, C6, and U373 cells and right side scale for C33A cells. (C) Mouse COUP-TF II promoter reporter plasmids −4000/*Bgl*II, −320/*Apa*I and RARβ–2-*lacZ* reporter plasmid were cotransfected with expression plasmid pRcCMVneo without any cDNA (control and dBcAMP+RA) or containing mCOUP-TF I (dBcAMP+RA+COUP-TF I) or mCOUP-TF II (dBcAMP+RA+COUP-TF II) cDNA into PCC7 cells. Cells were treated with all-*trans* RA (0.5 μM) and dBcAMP (1 mM) and CAT and *lacZ* activities were measured. Left side scale represents CAT activity for −4000/*Bgl*II and −320/*Apa*I constructs and right side scale represents *lacZ* activity for for RARβ–2-*lacZ* construct. The data shown are representative of at least three independent experiments.

the region −97 to −40. The putative HRE localized 68 nucleotides from the major start site is the only sequence that has certain homology to HRE in this region. Mutations in this HRE or deletion of this sequence do not affect inducibility of COUP-TF II promoter activity by RA. Also, coexpression of COUP-TF I or COUP-TF II, which completely blocks RA inducibility of RARβ2 promoter activity, only slightly reduces RA inducibility of COUP-TF II promoter activity. These data suggest that the effect of RA on COUP-TF II promoter activity is not directly mediated by the binding of RARs or RXRs to the response element, but is indirect. This hypothesis is supported by the data which demonstrate that the induction of the COUP-TF II gene expression by RA in teratocarcinoma P19 cells is a relatively slow process. Induction of COUP-TF II gene expression occurs 24–40 h after treatment of P19 cells with all-*trans* or 9-*cis* retinoic acid [19,34]. In contrast, induction of genes that are directly regulated by the RARs or RXRs, for example RARβ and midkine [31,35,36], occurs in less than 5 h.

Analyses of COUP-TF II promoter activity in different cell lines revealed that all-*trans* RA either stimulates, suppresses or has no effect on transcription. These results suggest that COUP-TF II is involved at least in two different functions of retinoids. First, in cells where COUP-TF II promoter activity is stimulated by retinoids, it may function as a part of the negative feedback loop to suppress effects of retinoids. Second, in cells where COUP-TF II promoter activity is suppressed by retinoids, the retinoid response may be long lasting. At the same time, COUP-TF II may also be involved in timing the switches of retinoic acid mediated gene regulation. Detailed analyses of regulation of COUP-TF II expression in different cell types, and characterization of its role in cell type specific gene regulation are required to understand the logic of COUP-TF II functioning during development and in the adult organisms.

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