Constitutive Activation of Retinoic Acid Receptor β2 Promoter by Orphan Nuclear Receptor TR2*

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The orphan nuclear receptor TR2 functions as a constitutive activator for the endogenous retinoic acid receptor β2 (RARβ2) gene expression in P19 embryonal carcinoma cells and for reporters driven by the RARβ2 promoter in COS-1 cells. The activation of RARβ2 by TR2 is mediated by the direct repeat-5 (DR5) element located in the RARβ2 promoter. Furthermore, cAMP exerts an enhancing effect on the activation of RARβ2 by TR2, which is mediated by the cAMP response element located in the 5′-flanking region of the DR5. The constitutive activation function-1 (AF-1) of TR2 is mapped to the CREMτ and TR2, detected by co-immunoprecipitation, which is mediated by the N-terminal AB segment of TR2. In gel mobility shift assays, TR2 competes with P19 nuclear factor binding to the RARβ2 promoter, and TR2 and CREMτ bind simultaneously to this DNA fragment. The role of TR2 in the early events of RA signaling process is discussed.

Nuclear receptors comprise a super family of transcription factors that contain a zinc finger-type DNA binding domain (DBD) and a ligand binding (LBD) domain and are able to regulate gene expression in a wide variety of biological processes (1-6). Some of these nuclear receptors are known hormone receptors, whereas the vast majority of the cloned nuclear receptors remain as orphan members. Despite the lack of identified ligands, the physiological roles of several orphan receptors have begun to be revealed in genetic studies (7-10).

The mouse orphan receptor TR2 was isolated from an E8.5 embryonic cDNA library (11), and the gene was characterized (12). The human TR2 was cloned from a prostate cDNA library (13). Like many other orphan receptors, the biological activity of TR2 was first demonstrated in several reporter systems. For instance, TR2 repressed reporters driven by a direct repeat (DR)-4 hormone response element derived from the mouse cellular retinoic acid-binding protein I gene promoter (14) and a DR1-type RA response element derived from the cellular retinol-binding protein II gene promoter (15). Moreover, TR2 strongly suppressed RA induction of a reporter driven by the DR5 derived from RA receptor β2 (RARβ2) promoter, mediated by competitive binding of TR2 to this DR5 (16-18). By using this DR5 reporter as a model system, the functional characteristics of TR2 were examined, and its molecular features required for a suppressive activity were revealed. It was demonstrated that the suppressive activity required the DBD, the ability to dimerize, the LBD, and two consecutive glutamate residues at amino acid (aa) positions 553/554 (16). In addition, a novel receptor heterodimeric pathway was identified that involved heterodimers of TR2 and TR4 (19). Recently, the mouse nuclear receptor-interacting protein 140 (RIP140) was cloned and demonstrated as a co-repressor for TR2 by interacting with its LBD (20).

In all these functional studies, TR2 appears to affect RA signaling pathways by regulating the components for RA metabolism (cellular retinoic acid-binding protein-I and cellular retinol-binding protein-II) and modulating RA induction of target gene expression. Of most significance is the potent suppression of RA induction of DR5-type RA response element derived from the RARβ2 gene promoter. RARβ2 is known as one of the earliest RA-responding genes in several culture systems, most notably the embryonal carcinoma cell cultures such as P19 and F9 (21-24). This gene serves as one of the master regulators in many RA-induced cellular events, such as proliferation, differentiation, and apoptosis, by regulating a number of downstream effector genes (25, 26). The RARβ2 gene is weakly expressed in stem cell populations and is rapidly induced by RA (27). A functional promoter of this gene consists of several essential DNA response elements spread in close proximity. These include, among others, an RA-responding DR5, a cyclic AMP-response element (CRE), and a TATA box (28). The DR5 is responsible for a rapid and potent RA induction, mediated by holo-RAR/RXR binding (21, 23, 29). The biological effects of cAMP has also been confirmed in the P19 culture model (28).

Although RA induction appears to be the most effective trigger that induces this gene expression in the stem cell cultures, the regulation of this gene in animals appears to be rather complicated. For instance, the expression pattern of this gene in animal tissues does not always correspond to the panel of tissues that are rich in RA (22, 30-32). Furthermore, this promoter cannot be activated by RA in a number of cell types such as breast tumor and lung cancer cell lines as well as some pituitary cell lines (33-35) despite the detection of a potent RA induction in these cells using reporters containing the dissected DR5 (34, 36). All these observations suggest that the induction of the RARβ2 gene may involve factors other than RA.

Like the studies of several other orphan receptors CUP-TF, nerve growth factor-1B, Dax1, and hepatocyte nuclear factor-4 (37-40), previous studies of TR2 in different labs have utilized...
RA response element- and other hormone response element-containing reporters to examine its biological activities (5, 24, 25, 28, 29). The finding that apo-TR2 is able to bind to this DR5 with a high affinity (an estimated $K_d$ of 4–7 nM) in the absence of putative ligands (12, 16, 17) has prompted us to examine the effect of TR2 expression on the endogenous RAR$_{a2}$ gene activity in the absence of RA. In this study, it is demonstrated that apo-TR2 functions as a constitutive activator for reporters driven by the RAR$_{a2}$ promoter containing the DR5 element, and overexpression of TR2 in P19 stem cells activates the endogenous RAR$_{a2}$ gene expression. Furthermore, cAMP exerts an enhancing effect on the activating function of TR2 on cAMP-response element-binding protein (CREB), CREM$_T$ (41). Finally, in gel mobility shift assays, TR2 competes with P19 nuclear factors binding to the RAR$_{a2}$ promoter, and TR2 and CREM$_T$ bind simultaneously to this DNA fragment. We now report these studies characterizing TR2 as a constitutive activator for the RAR$_{a2}$ promoter, which can be enhanced by cAMP.

### EXPERIMENTAL PROCEDURES

**Construction of Reporters and Expression Vectors**—The RAR$_{a2}$ reporters were constructed by fusing genomic sequences generated by polymerase chain reactions (PCRs) (for CRE/DR5/TATA-luc and ~CRE/DR5/TATA-Luc), SmaI digestion (for DR5/TATA-luc), or re-anneling of oligonucleotides (for TATA-luc) upstream to a promoterless luciferase cDNA, pGL3 (Promega, Madison, WI). The cDNA for mouse CREM$_{T}$ (42) was also obtained in a reverse transcription-converted PCR (RT-PCR) using mouse testis mRNA as the template and confirmed by DNA sequencing. The cDNA of CREM$_T$ was cloned into the pSG5 vector at BgIII site for expression in mammalian cells and for in vitro transcription/translation reactions (for gel mobility shift assay). The expression vectors for TR2 and deletions and point mutations, cloned in pSG5 vectors, were described previously (16). The dissected TR2 segments to be cloned into mammalian two-hybrid expression vectors (see "Mammalian Two-hybrid and Transactivation Assays" under "Experimental Procedures") were obtained by either restriction digestion of TR2 cDNA or PCR. The fragment containing the AB domain and a small portion of the zinc finger (aa 1–138) was obtained as a 0.4-kilobase fragment by EcoRI/PstI digestion at the N terminus of TR2 cDNA, the A segment (aa 1–30) was obtained as a 0.15-kilobase fragment by EcoRI/BamHI digestion, and the B segment (aa 5138) was obtained by BamHI/PstI digestion in a size of 0.25 kilobases. Further deletions of the A domain (A-1/40, 1/30, 10/30, and 10/25) were obtained in PCRs. Table I summarizes the oligonucleotides used in PCRs to generate specific DNA fragments for this study. All PCR-generated DNA fragments have been confirmed by DNA sequencing.

### Cell Culture and Transfection Techniques—The P19 cell line, maintained as described previously (43), was used to determine the effects of expressing TR2 on the endogenous RAR$_{a2}$ gene activity. COS-1 cells were used in co-transfection experiments to determine the effects of TR2 on nuclear receptors on the reporter gene activity as well as mammalian two-hybrid interaction experiments as described (16, 20). All the COS-1 cultures were maintained in Dulbecco’s modified Eagle’s medium containing dextran charcoal-treated serum (DCC medium). Transfection was conducted by using the calcium phosphate precipitation method, and LacZ and luciferase activities were determined as described (16). The luciferase activity was normalized to the LacZ activity by the internal control to obtain specific luciferase unit. To compare the relative activity of different expression vectors on the reporter, the specific luciferase unit of the control vector was assigned an arbitrary value of 1, in order to determine the relative luciferase unit of each expression vector, which represented the relative activity of the expression vector. Triplicate cultures were used in each transfection experiment, and three independent experiments were conducted to obtain the means and S.E. for all the transfection experiments.

**RT-PCR to Detect Endogenous RAR$_{a2}$ Expression**—RT-PCR was conducted to detect the expression of endogenous gene expression in P19 cultures. Primers for RAR$_{a2}$ were 5’-TGGACCTTTTCTGTGCGGC-3’ (nucleotide position 391–408 in Fig. 2 of Ref. 24), where the 5’-untranslated region of RAR$_{a2}$ is located, and 5’-GGGAATGTCTGCAACAGCT-3’. The PCR reaction cycle was 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, for a total of 30 cycles. Five µl of each PCR product was analyzed on Southern blots and probed with actin- and RAR$_{a2}$-specific probes.

**Mammalian Two-hybrid Interaction and Transactivation Assays**—For the mammalian two-hybrid interaction assay, mP (containing the DBD of GAL4) and cP (containing the activation domain of VP16) (CLONTECH, Palo Alto, CA) were used to construct the expression vectors. Pairs of mP and cP fusions were tested in parallel. To examine intermolecular interactions, the cDNA for CREM$_T$ was cloned into mP and tested against TR2 fragments cloned in cP. The reporter construct for the mammalian two-hybrid interaction as well as transfection procedures were as described previously (20).

To detect the intrinsic transactivator function of TR2, various TR2 segments were fused to mP vector. A GAL4 binding site-driven luciferase reporter and the internal control lacZ were as described (20). Activation of reporter by GAL4 fusions was determined by comparing their specific luciferase units to that of the control.

**Co-immunoprecipitation Assay**—The anti-TR2 antibody generated previously (12, 17) was not effective for immunoprecipitation; therefore, we utilized a hemagglutinin (HA)-tagged TR2 expression vector (20) in immunoprecipitation experiments. The biological activity (suppression of RA induction on the DR5-containing reporter) and biochemical properties (binding to DR5 element) of HA-TR2 expressed from this vector...
were demonstrated previously (20). The activation function of HA-TR2 on RARβ2 promoter was confirmed in this study (see “Results”). This vector was used to transfect COS-1 cells in co-immunoprecipitation experiments involving TR2. COS-1 cells were transfected with the HA-TR2 in the presence or absence of the CREM expression vector. Cells from one 10-cm plate for each treatment were harvested at 36–48 h and resuspended in 200 μl of lysis buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, 1 mM dithiothreitol, 2 μM phenylmethylsulfonyl fluoride, 10% glycerol, protease inhibitor mixture). For immunoprecipitation, 100 μl of the cell lysate was incubated with a mouse anti-HA monoclonal antibody (Roche Molecular Biochemicals) at 4 °C for 2 h, followed by the addition of 15 μl of protein G-Sepharose CL4B resin (Sigma). The precipitation was conducted overnight at 4 °C, and the resin was vigorously washed three times with the lysis buffer and resuspended in a SDS-polyacrylamide gel electrophoresis loading buffer for Western blot analysis. A 10% polyacrylamide gel was used for protein separation, and the gel was transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The blot was incubated with a rabbit anti-CREM antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight, followed by washing and reaction with a mouse anti-rabbit secondary antibody and detection with ECL (Amersham Pharmacia Biotech). Nuclear extract of P19 was prepared as described previously (20). Nuclear extract of P19 was isolated as described (44). The extract from a 10-cm dish was resuspended in a volume of 60 μl and 4 μl (about 10 μg of nuclear protein) was used in each gel shift reaction. Protein was also prepared using in vitro transcription and translation reactions (TNT, Promega). For protein-DNA interactions, the in vitro translated protein or nuclear extract isolated from P19 cells was incubated with 1 ng of probe in a 20 μl of binding buffer. The probes were prepared by labeling the double-stranded DNA fragments isolated from the genomic segments described in a previous section (CRE/DR5/TATA and DR5/TATA) with [γ-32P]dCTP using Klenow enzyme. A fragment containing only the DR5 (16) was also prepared by annealing oligonucleotides spanning DR5 site and used as a control for TR2 binding.

**RESULTS**

**Constitutive Activation of RARβ2 by TR2 Expression**—Previously, the biological activity of orphan receptor TR2 was demonstrated to be primarily suppressive on both RA induction of DR5-containing reporters (16, 17) and reporters regulated by some hormone response elements such as DR4 (14) and DR1 (15). It was demonstrated that the suppressive activity required the intact LBD and DBD but not the N-terminal A/B domains of the molecule (16). In addition, the suppressive effects of TR2 on RA induction of DR5-driven reporter was attributed to its competitive binding to DR5 (16, 18). In an attempt to determine the constitutive activity of TR2 on DR5 reporters, i.e., its biological activity in the absence of RA, we set up experiments to examine the effects of TR2 expression on RARβ2 gene activities using both reporter and endogenous gene expression systems.

To examine the effects of TR2 expression on the endogenous RARβ2 gene expression, we used P19 stem cells, which expressed a negligible level of TR2 and RARβ2 (18), as an experimental system. P19 cells maintained in medium containing charcoal-treated serum were induced with RA or transfected with the wild type TR2 expression vector. RNA was isolated 12 or 24 h after the addition of RA or transfection. To detect RARβ2 mRNA specifically, RT-PCR was conducted to examine its mRNA level. As shown in Fig. 1, the expression of RARβ2 (top panel) increases as compared with actin (lower panel) in cells either transfected with TR2 (lanes 2 and 3) or induced with RA (lane 1). As expected, the control culture (lane 6) expresses no detectable RARβ2 under this condition. Although treating the cells with cAMP alone has no effects on RARβ2 expression at 12 h (lane 4), transfection with TR2 for 12 h in the presence of cAMP results in a further enhanced RARβ2 expression (comparing lanes 2 and 5).

To further demonstrate the biological activities of TR2 on RARβ2 promoter, we constructed a luciferase reporter driven by a contiguous regulatory region of this promoter, a 91-base pair genomic segment containing the CRE followed by the DR5 element and TATA box (−100 to −10, Ref. 24). This reporter was designated as CRE/DR5/TATA-luc. The effects of TR2 expression on this reporter was assessed in transfected COS-1 cells supplemented with charcoal-depleted serum. As shown in Fig. 2, TR2 expression activates this reporter activity in a dose-dependent manner (filled columns). For a control, a mutant deleted in the most N-terminal A segment (TR2-ΔA) has been included and shown to be inactive in this assay (open columns). To confirm the activation function of HA-TR2, which was to be used in co-immunoprecipitation experiments, this expression vector was also examined in parallel experiments (striped columns). As shown in the same figure, TR2 tagged with an HA epitope remains as effective as the wild type protein in terms of the activation function in this reporter system.

Collectively, these data indicate that TR2 encodes a constitutive activation function for the endogenous RARβ2 gene as well as the reporter driven by its promoter in the absence of putative ligands. Furthermore, the activation of RARβ2 by TR2 is enhanced by cAMP.

**Domains of TR2 Required for Its Activation Function**—To determine the molecular domains required for this novel activation function of TR2, a panel of TR2 deletion mutants generated previously (16) that have been shown to express well and localize properly were first used to determine the required molecular features of TR2 as an activator for the CRE/DR5/TATA reporter. As shown in Fig. 3, deletions from either the N- (TR2-2A) or the C terminus (−20, −50, −100, and −200) completely abolishes this activation function (columns 3–7), indicating that the activation function of TR2 requires an intact molecule. In addition, point mutations that abolish dimerization...
and DNA binding (LLL mutant, column 9) or affect the putative AF-2 domain conformation (EE mutant, column 8) are also defective in this activation function. Therefore, the activation function of TR2 on RARβ2 promoter requires an intact receptor including the N-terminal domain, the DBD, and the LBD.

**Defining the AF-1 Transactivation Domain**—To define the activation domain of TR2 and to examine whether TR2 encoded an intrinsic, transactivation function, the entire TR2 as well as its dissected portions was each fused to pM for the test of a transactivation activity as described under “Experimental Procedures.” The intact TR2 (TR2-f), its LBD (aa 166–590), the putative AF-2 (aa 570–590), the AB domain with a small portion of the zinc finger (aa 1–138), and the dissected A domain (aa 1–50) or B domain (aa 51–138) were first individually fused to the same pM vector and assessed in transactivation tests. As shown in Fig. 4, neither the intact TR2, the LBD, the putative AF-2, the AB, or the B domain is able to transactivate the GAL4 DBD (columns 1–5 and 11). Interestingly, the fusion of A domain alone to the GAL4 DBD dramatically induces GAL4 reporter (column 6), indicating that the dissected A domain encodes an activation function that can be transferred to a heterologous molecule such as the DBD of GAL4. However, this activation function may be masked in the context of the AB segment, since AB segment does not transactivate the reporter (column 4). To further dissect the minimal sequence required for such a transactivation function, more deletions were made in the A domain. As shown in the same Fig. 4 (columns 7–10), the A domain that retains aa 1/40, 1/30, or 10/30 (columns 7–9) remains active in this assay, whereas a further 5-aa deletion from the smallest pM-10/30 fragment (leaving only aa 10 to 25, pM-10/25, column 10) abolishes this activity completely. Therefore, the transferable activation function (AF-1) of TR2, as demonstrated in a GAL4 fusion, requires only a small segment (aa 10 to 30) of the N-terminal A domain.

**The Effects of cAMP**—Studies described in Fig. 1 show that TR2 constitutively activates the RARβ2 promoter activity, which can be enhanced by cAMP. In the RARβ2 promoter, a CRE is located approximately 40 base pairs upstream of the DR5 element, and cAMP is known to enhance RA induction of

**Fig. 3. Mapping of the domain required for the activation function of TR2.** A panel of TR2 deletion vectors (250 ng each) constructed previously (16) were tested in transfection experiments as described in Fig. 1. The major deletions and mutations are indicated on the TR2 map shown above the figure. The specific luciferase unit of the control vector (column C) was arbitrarily assigned a value of 1 to obtain relative luciferase units (RUL) of each expression vector.

**Fig. 4. Transactivation function of the A domain of TR2.** Different segments of TR2 were fused to the DBD of GAL4 (pM), and the ability of each segment to transactivate a GAL4-luciferase reporter was determined. A, all the GAL4-TR fusion constructs. Numbers above each map indicate the aa positions of each TR2 segment. B, the specific activity of each construct, represented as the specific luciferase unit.
Fig. 5. The effect of cAMP on the activation function of TR2. A, four RARγ2 reporters were generated as described under “Experimental Procedures,” and the relative positions of these regulatory elements in the RARγ2 promoter are indicated. B, the effects of TR2 and cAMP on the expression of four RARγ2 reporters. COS-1 cells were transfected with the vectors indicated (C for control, TR2 for the wild-type TR2 and treated with vehicle (−) or cAMP (+)). Specific luciferase units were determined at 24 h. By using the control activity (C) as 1 in each group, the relative activity (RLU) of each treatment in the same group was determined. The open bars show the group using CRE/DR5/TATA-luc as the reporter, the shaded bars show the group using −CRE/DR5/TATA-luc as the reporter, and the black bars show the group using TATA-luc as the reporter.

Fig. 6. Intermolecular interaction of TR2 with CREMγ. A, co-immunoprecipitation of TR2 and CREMγ. Immunoprecipitation experiments were conducted as described under “Experimental Procedures.” The precipitated TR2 is indicated with a single arrow (labeled with CREMγ). The precipitated CREMγ or CREM-like protein is indicated with a double arrow (labeled with CREMγ). Lane 1 shows the result of COS-1 cotransfected with TR2 and a CREMγ expression vector. Lane 2 shows the result of COS-1 transfected with the TR2 expression alone, and lane 3 shows a control experiment without the expression vectors. B, mammalian two-hybrid interaction tests. The interaction of CREMγ (cloned in pM vector) with various portions of TR2 cloned in pVP vector was examined in the mammalian two-hybrid interaction test as described under “Experimental Procedures.”
was stripped off the blot, which was subsequently detected with a rabbit anti-TR2 antibody as shown in the lower panel.

To determine which portion of TR2 molecule interacted with CREM, mammalian two-hybrid interaction tests were performed. The CREM-coding region was fused to the pM vector and tested against various portions of TR2 fused to the pVP vector. COS-1 cells were co-transfected with pairs of pM and pVP fusions together with the GAL4 reporter and an internal control lacZ vector. As shown in Fig. 6B, two pairs of control vectors induce only a background level of reporter activities (columns 1 and 2), and the cloned CREM interacts strongly with the AB segment (column 4) but not the LBD (column 5). Interestingly, deleting the B domain from the AB segment dramatically reduces this interaction (comparing columns 3 and 4), and the B domain alone fails to interact with CREM (column 6). Therefore, CREM is able to interact with the AB domain of TR2, and the B domain affects the ability of TR2 to interact efficiently with CREM.

**DNA Binding Properties of TR2 and CREM on RAR\(\text{beta}_2\) Promoter**—Our previous studies demonstrated a specific binding of TR2 to the dissected DR5 of RAR\(\text{beta}_2\) promoter, and the binding affinity was estimated to be approximately 7.4 nM to the dissected DR5 DNA fragment (16). Since the expression of TR2 activated the endogenous RAR\(\text{beta}_2\) expression in P19 cells (Fig. 2), it was of interest to compare the binding of TR2 and P19 nuclear factors to this sequence in its genomic context. Gel shift experiments were conducted to examine P19 nuclear factors binding and TR2 binding patterns on the DR5-TATA fragment of RAR\(\text{beta}_2\) promoter. As shown in Fig. 7A, consistent with our previous studies in which an isolated DR5 probe was used (16), one major retarded band appears in the reaction using in vitro translated TR2 alone (lane 2) that can be competed efficiently by a 20-fold excess of cold fragments (indicated with a double arrow on the left of lane 1). Interestingly, P19 nuclear factors bind to the same fragment in a very different pattern, characterized by four differentially migrating bands (indicated with four arrowheads on the right of lane 6). The slowest migrating fragment can be competed very efficiently by a merely 4-fold excess of cold fragments (lane 5), whereas the other three bands are competed less efficiently, with an approximately 50% competition by a 20-fold excess of cold fragments (lane 3). To compare the preference of this sequence with regard to TR2 or P19 nuclear factors binding, we have performed another gel shift experiment to examine competitive binding of TR2 and P19 factors as shown in Fig. 7B. Consistent with results shown in Fig. 7A, TR2 binding to this sequence results in a single retarded band (lane 4, indicated with double arrow), and P19 nuclear factors binding results in four differentially migrating bands (lane 3, indicated with arrowheads). However, in the presence of TR2, the P19-specific bands dramatically reduce in the intensity, whereas the TR2-specific band remains strong (lane 2). This result indicates that TR2 strongly competes with P19 endogenous nuclear factors in binding to this promoter.

The finding that TR2 was able to interact with the cloned CREM and cAMP enhanced the activation of RAR\(\text{beta}_2\) by TR2 prompted us to examine the DNA binding patterns of TR2 and CREM. A gel shift experiment was then conducted by using the contiguous CRE/DR5/TATA segment as the probes. As shown in Fig. 7C, TR2 alone binds to this fragment, shown as a major retarded band (lane 5, single arrow). Similarly, CREM alone also binds to this sequence, shown as a slightly faster migrating band (lane 3, small arrow head). In the presence of both TR2 and CREM, a super-shifted band appears (lane 2, double arrow), indicating that TR2 and CREM together are able to bind to this sequence at the same time. Interestingly, the addition of an anti-CREM antibody abolishes the super-shifted band but not the TR2 band (lane 4), indicating that this antibody alters the conformation of CREM, thereby affecting its interaction with DNA or TR2. Therefore, TR2 and CREM not only are able to interact directly with each other as demonstrated in two-hybrid interaction and immunoprecipitation assays but also can si-
TR2 as a Constitutive Activator for RARβ2

This study demonstrates for the first time a constitutive activation function of orphan nuclear receptor TR2 on the endogenous RARβ2 gene expression in P19 cells as well as reporters driven by the promoter of this gene. The activation is mediated by the DR5 element, which can be enhanced by cAMP through the upstream CRE element. The activation domain of TR2 was mapped to aa 10–30 in its N-terminal A segment. Intermolecular interaction occurs between TR2 and CREMβ, as demonstrated in two-hybrid interaction and co-immunoprecipitation assays. The molecular interaction of TR2 with CREMβ is mediated by the N-terminal AB segment. On the RARβ2 promoter, TR2 and CREMβ bind simultaneously to the DNA, and TR2 competes efficiently with P19 nuclear factor binding to this promoter.

The nature of TR2 as an activator exhibits two features. First of all, the activation is specific to RARβ2 promoter, since TR2 represses other promoters that also contain a response element for TR2 such as the cellular retinoic acid-binding protein-I or SV40 promoters (data not shown). Although cAMP is able to enhance the activation of RARβ2 by TR2, the intrinsic activity of TR2 cannot be attributed solely to the cAMP pathway, since the RARβ2 promoter deleted in the CRE can still be activated by TR2 but at a lower level. We have failed to detect any interaction of TR2 with other potential co-activators such as TBP or SRC-1 in either immunoprecipitation or two-hybrid interaction tests (data not shown). Therefore, the biochemical basis of this activation function of TR2 remains to be determined.

Nevertheless, a physiological connection between these two pathways remains to be further established by genetic tests.

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