Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2718–2722, April 1992
Biochemistry

RAR-β4, a retinoic acid receptor isoform is generated from RAR-β2 by alternative splicing and usage of a CUG initiator codon

(retinoic acid-inducible/non-AUG initiator codon/specific expression/development)

SUNIL NAGPAL, ARTHUR ZELENT, AND PIERRE CHAMBON

Laboratoire de Génétique Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique Unité 184 de Biologie Moléculaire et de Génie Génétique de l'Institut National de la Santé et de la Recherche Médicale Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg Cedex, France

Contributed by Pierre Chambon, December 23, 1991

ABSTRACT **An isoform of retinoic acid receptor β, RAR-β4, has been identified. RAR-β4 is expressed under the control of the same retinoic acid-responsive promoter as RAR-β2. RAR-β4, which is generated by alternative splicing from the same primary transcripts as RAR-β2, is initiated by a non-AUG codon, CUG. The amino acid sequence of RAR-β4 in regions B–F is identical to that of the other RAR-β isoforms β1, β2, and β3. However, the RAR-β4 A region is much shorter (4 amino acids long) than those of these isoforms. RAR-β4 exhibits a tissue-specific pattern of expression and distinct transcriptional activation properties when compared with the other RAR-β isoforms.**

Cloning of cDNAs encoding the three mouse and human retinoic acid receptors (RARs) α, β, and γ, which belong to the steroid/thyroid hormone receptor superfamily of ligand-dependent transcriptional transregulatory factors, has markedly contributed to our understanding of the mechanism through which RA exerts its highly polymorphic effects during development and in adult vertebrates (for reviews, see refs. 1 and 2). Like the other members of this superfamily, RARs have a modular organization composed of six regions of different evolutionary conservation (regions A–F; see Fig. 1*A* and ref. 3 for a review). Regions C and E are responsible for DNA and ligand binding, respectively (see refs. 3–6 for reviews). In the case of steroid hormone receptors, cell- and promoter-specific transcriptional activation functions (TAFs) are associated with the A/B and E regions, and the activity of the TAF located within region E has been shown to be ligand dependent (3, 6–8). A similar TAF has been characterized in the E region of RARs (M. Saunders and P.C., unpublished results). The functional properties of the D and F regions are still unknown and, in contrast to the steroid hormone receptors, no independent TAF has been identified in RAR A/B regions.

A number of mRNA isoforms are generated from each RAR gene (either α, β, or γ) by differential usage of two promoters and differential splicing of the primary transcripts. For both RAR-α (9) and RAR-γ (10, 11) genes, two main and several minor isoforms have been characterized, which in general share B–F sequences but differ in the sequence of 5′ untranslated region (5′-UTR) and N-terminal A regions. In the case of the RAR-β gene, three isoforms have been identified (12). RAR-β1 and RAR-β3 are generated by alternative splicing of a primary transcript initiated from the more 5′ upstream promoter P1 (ref. 13; C. Mendelsohn, S. Larkin, A.Z., and P.C., unpublished results), whereas RAR-β2 is derived from a primary transcript initiated from the downstream promoter P2, which contains a RA-responsive element (refs. 12–15 and references therein). Interestingly, the degree of interspecies conservation of the amino acid sequence of regions B–F of a given RAR type (α, β, or γ) is higher than that of the same regions of all three RARs within a given species (see ref. 2 for review). Moreover, the 5′-UTR and the A region sequences, which are specific for each RAR isoform, are also highly evolutionarily conserved (1, 9, 11, 12). These conservations, together with specific developmental patterns of expression (refs. 2 and 16 and references therein) have suggested that the three RARs and their isoforms perform specific functions, thus accounting, at least in part, for the highly pleiotropic effect of RA during development and in the adult.

We report here the characterization of a mouse RAR-β (mRAR-β) isoform cDNA that corresponds to a mRNA generated by alternative splicing of the same primary transcript as mRAR-β2. Furthermore, we show that initiation of translation on this mRNA takes place at a CUG initiation codon, resulting in a mRAR-β4 protein, which has a very short (4 amino acids long) A region.

MATERIALS AND METHODS

mRAR-β4 CTG was constructed by deleting the *Eag* I/*Sac* I (nucleotides 244–653) fragment from mRAR-β2 (mRAR-βØ in ref. 17) and inserting a 56-base-pair (bp) synthetic *Eag* I/*Sac* I oligonucleotide containing sequences in tandem from nucleotides 244–265 and 620–653. Thus, the resulting clone contained a deletion of RAR-β2 nucleotides from positions 266–619. Similarly, mRAR-β4 TTG and mRAR-β4 ATG were constructed by using synthetic 56-bp *Eag* I/*Sac* I oligonucleotides in which the CTG initiator codon was mutated to TTG and ATG, respectively. All other materials and methods were as described (12, 17, 18).

RESULTS AND DISCUSSION

Cloning and Characterization of mRAR-β4 cDNA. mRAR-β4 cDNA was isolated by using polyadenylylated [poly(A)$^+$] RNA prepared from 8.5-day postcoitum mouse embryos and the anchored PCR technique as described (12, 19). The two nested B region-specific oligonucleotide primers that were used in two successive rounds of PCR were for the first round 5′-TAGGATCCTGGGCTCGGGACGAGC-TCCT-3′ (nucleotides 647–666) and for the second round 5′-ATGGATCCGAGCTCCTCAGAGCTGGTA-3′ (nucleotides 636–655) (see Fig. 1*C* and ref. 12; restriction enzyme site linker sequences are underlined). The resulting cDNAs were cloned and screened first with a ^{32}P-labeled oligonucleotide probe specific for the B region (nucleotides 620–636 in Fig. 1*C*) and then with oligonucleotide probes specific for the A regions of mRAR-β1, -β2, or -β3 (A1, A2, and A3 regions, respectively; see ref. 12) (data not shown). Two clones that

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RA, retinoic acid; RAR, RA receptor; mRAR, mouse RAR; 5′-UTR, 5′ untranslated region; ORF, open reading frame; tk, thymidine kinase; CAT, chloramphenicol acetyltransferase.

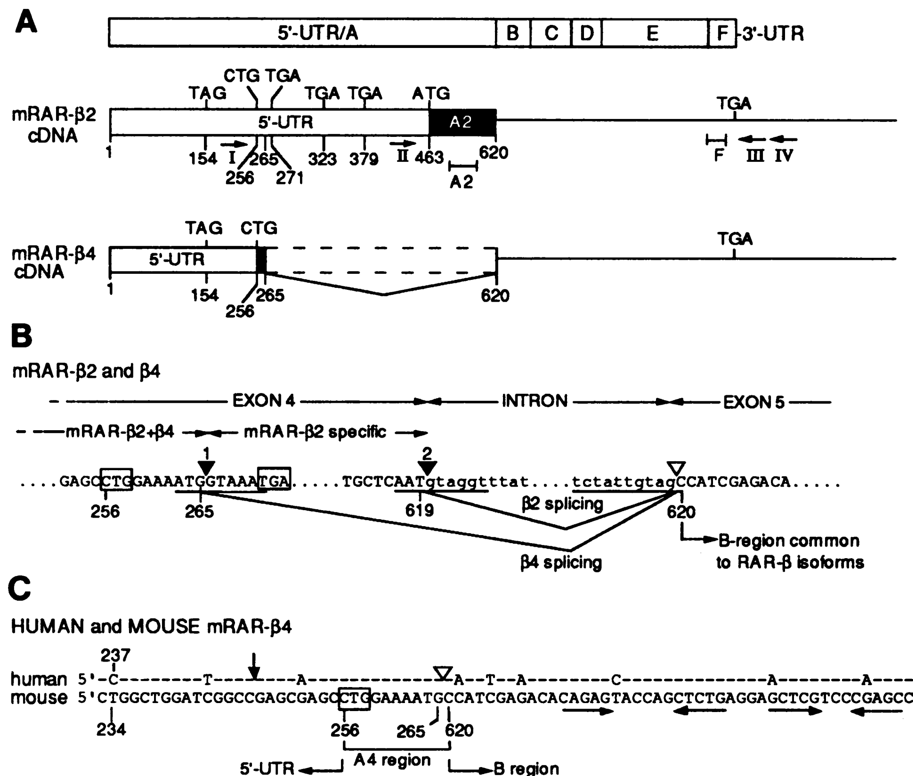

FIG. 1. (*A*) Schematic representation (not to scale) of mRAR-β2 and mRAR-β4 cDNA isoforms. General organization of the RAR sequence is shown at the top and the two isoforms are schematized below. Location of sequences that encode regions B–F and the 3′-UTR common to all RAR-β isoforms (1, 2, 12, 17) is indicated as well as those of the 5′-UTR and A region, which are isoform specific. Numbers correspond to nucleotide positions in the mRAR-β2 cDNA sequence (12). Sequences of mRAR-β2 and -β4 lying upstream of the A/B region junction are indicated with an open box. The A regions of mRAR-β2 (A2) and mRAR-β4 (A4) are represented as solid boxes. The region of mRAR-β2 that is spliced out to give mRAR-β4 (nucleotides 266–619) is indicated with a dashed line. Initiator and terminator codons are indicated. The CTG initiator codon (position 256) is shown on the mRAR-β4 sequence. Arrows indicate the approximate location of oligonucleotide primers used for reverse transcription (IV) and PCR (I–III). Synthetic oligonucleotide primers II–IV have been described (12) and oligonucleotide primer I corresponds to nucleotides 239–259 of the mRAR-β2 sequence. The approximate locations of probes A2 and F (12) are also indicated. (*B*) Sequence and schematic representation of donor splice sites 1 (position 265) and 2 (position 619), whose alternate utilization with the acceptor site located at the 5′ end of the B region-encoding exon [exon 5, previously exon 4 (12)] gives rise to mRAR-β4 and mRAR-β2 isoforms, respectively. Donor and acceptor splice sites are underlined. The mRAR-β4 CTG initiator codon and the downstream TGA terminator, which is present only in the mRAR-β2 sequence, are boxed. Sequences shown have been derived from previously described mRAR-β genomic clones that contained exon 4 (previously exon 3) and exon 5 (previously exon 4) as well as their adjacent intronic sequences (12). (*C*) Alignment of a mRAR-β4 partial cDNA sequence with the sequence of its putative human counterpart (see ref. 12). Only divergent nucleotides are indicated. The CTG initiator codon is boxed and horizontal arrows indicate downstream inverted repeats (see text). The sequence of the longest mRAR-β4 cDNA clone isolated by PCR is shown and the 5′ end of the shorter clone is indicated with a vertical arrow (see text). The A/B junction is indicated by an open arrowhead.

hybridized with the B region-specific probe, but not with A1, A2, or A3 region-specific probes, were isolated and sequenced. Both clones had identical sequences upstream of the A/B region junction, which were different from those of the A1, A2, and A3 region sequences (see Fig. 1*C*, in which the longer sequence is displayed and the 5′ end of the shorter one is indicated by an arrow; compare with A1, A2, and A3 sequences in ref. 12).

The above results indicated the possible existence of a fourth mRAR-β isoform that we called mRAR-β4. A comparison of its cDNA sequence upstream of the B region with the corresponding sequences of mRAR-β1, -β2, and -β3 isoforms (12) revealed a complete identity with the sequence located between nucleotides 232 and 265 in the 5′-UTR of mRAR-β2 (Fig. 1 *B* and *C*). Interestingly, the DNA sequence around nucleotide 265 corresponds to a nearly consensus splicing donor site 5′-(C/A)AG/GTRAGT-3′ (R, purine) (20) and is located within the exon (exon 4, formerly called exon 3 in ref. 12) that encodes the entire 5′-UTR and A regions of mRAR-β2 and immediately downstream from the RA-responsive β2 promoter (12, 13). Note that this splicing donor site (site 1 in Fig. 2*B*) is fully conserved in the human RAR-β2 sequence (12). Thus, mRAR-β4 appeared to be generated by an alternative splicing event occurring within the 5′-UTR of mRAR-β2 and resulting in the splicing of the first 265 nucleotides of this region to the first nucleotide (nucleotide 620) of exon 5, which encodes the mRAR-β B region (Fig. 1 *A* and *B*). That the first 265 nucleotides of mRAR-β2 and -β4 were identical was demonstrated by PCR using 5′ and 3′ oligonucleotide primers specific for both ends of the putative mRAR-β4 mRNA sequence located upstream of exon 5 (data not shown). Similar PCRs using 3′ oligonucleotide primers located in the 3′-UTR of the mRAR-β gene (Fig. 1*A*, primers III and IV) showed that mRAR-β4 mRNA also contains the sequence encoding the mRAR-β B–F regions common to all isoforms (data not shown, but see below).

Relative Abundance of mRAR-β2 and -β4 Transcripts. Two closely migrating RAR-β RNA species could be revealed by Northern blot analysis when using a ^{32}P-labeled cDNA probe corresponding to the entire 5′-UTR and A2 region of mRAR-β2, which suggested that the faster-migrating species may correspond to mRAR-β4 (Fig. 2*A*). This assumption was supported by the observation that a single RNA species was detected (Fig. 2*A*) when using a probe specific for the A2 region of mRAR-β2 (probe A2 in Fig. 1*A*; see ref. 12). A PCR study was then performed to clearly demonstrate the pres-

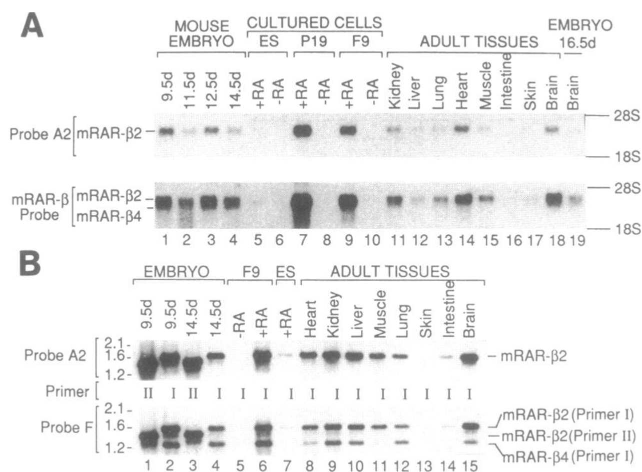

FIG. 2. mRAR-β2 and -β4 RNAs in mouse embryos, cultured cells, and adult tissues. (*A*) Northern blot analysis using ^{32}P-labeled probes corresponding to oligonucleotide A2 (see text and ref. 12) (*Upper*) or the cDNA corresponding to the whole 5'-UTR and A2 region of mRAR-β2 (*Lower*). The probes were labeled to a specific activity of $\approx 10^9$ cpm per μg of DNA. Either 15 μg of total RNA from ES cells treated and untreated with RA (1 μM) (lanes 5 and 6, respectively), or 4 μg of poly(A)$^+$ RNA (all other samples are as indicated above each lane; lanes 1–4 and 7–19) was used for Northern blot analysis as described (12). The positions of mRAR-β2 and mRAR-β4 mRNAs are indicated on the left and those of 28S and 18S RNAs are indicated on the right. (*B*) PCR analysis of mRAR-β2 and -β4 transcripts in various mouse tissues and cell lines, as well as in 9.5- and 14.5-day embryos. Experimental design and all conditions were as described in the text and in ref. 12. The figure corresponds to a Southern blot of PCR-amplified cDNAs (using primer I or II) hybridized with ^{32}P-labeled oligonucleotide probes specific for either the A2 region of mRAR-β2 (probe A, *Upper*) or the F region common to both isoforms (probe F, *Lower*). Identity of the 5' oligonucleotide primers (see Fig. 1*C*) used for amplification of either mRAR-β2 or -β4 cDNA in a given sample (as indicated above each lane) is designated with Roman numerals. The mRAR-β2 and -β4 species of expected size (see text) are identified on the right and the relative positions of molecular size markers (in kilobases) are indicated on the left.

ence of mRAR-β4 transcripts and to analyze the relative abundance of mRAR-β2 and -β4 mRNA isoforms. Poly(A)$^+$ RNA prepared from mouse embryos, cultured cells, and adult mouse tissues [total RNA was used in the case of embryonic stem (ES) cells] was first reverse transcribed by using Moloney murine leukemia virus reverse transcriptase and the 3' primer IV (Fig. 1*A*, see ref. 12). The resulting cDNAs were then PCR amplified by using the 3' primer III (Fig. 1*A*) and either the 5' primer II, which is specific for mRAR-β2, or the 5' primer I, which is common to mRAR-β2 and -β4 5'-UTRs (Fig. 1*A*). When the latter primer was used, two cDNA species, whose sizes corresponded to those expected for mRAR-β2 and mRAR-β4 (1608 and 1463 bp, respectively), were clearly seen when the PCR cDNA products were revealed with an F region-specific probe (probe F in Figs. 1*A* and 2*B*). On the other hand, only one major cDNA species was detected when the 5' primer was primer II, which is specific for mRAR-β2 (Fig. 2*B*). As expected, only the mRAR-β2 upper species was seen when the mRAR-β2-specific oligonucleotide A2 probe was used (Figs. 1*A* and 2*B*).

mRAR-β2 and -β4 transcripts were expressed at comparable levels in day 9.5 and 14.5 embryos and in F9 embryonal carcinoma cells, whereas RAR-β4 mRNA was less abundant than RAR-β2 mRNA in ES cells (Fig. 2*B*). As expected from their generation from the same RA-responsive promoter, the presence of both mRAR-β2 and -β4 transcripts was dependent on addition of RA to F9 cell growth medium. Similar levels of mRAR-β2 and -β4 transcripts were also found in several adult mouse tissues (kidney, liver, lung, and skin; Fig. 2*B*; data not shown), whereas mRAR-β2 mRNA was clearly more abundant than its mRAR-β4 counterpart in heart, muscle, intestine, and brain. These tissue-specific differences suggest the existence of tissue-specific differential splicing mechanism(s) and/or factors differentially regulating the stability of the two isoform RNA species. We note, in this respect, that the sequences of the splice donor sites 1 and 2 are closely related (Fig. 1*B*) and that the 5'-UTR, which is specific to the RAR-β2 isoform, is highly conserved between human and mouse, particularly in the sequences corresponding to the two small open reading frames (ORFs), sORF2 and sORF3 (see ref. 12). This suggests that these 5'-UTR sequences may play a specific function(s) in the regulation of mRAR-β2 mRNA stability and/or efficiency of translation (see ref. 12 for further discussion on this point). In contrast, the 5'-UTR, which is common to both β2 and β4 isoforms, is less conserved when the human and mouse sequences are compared, although both contain the small ORF, sORF1 (see ref. 12).

Translation of mRAR-β4 mRNA Is Initiated from a CUG Codon. There is no AUG initiator codon located upstream from the A/B region junction in the mRAR-β4 mRNA isoform [Fig. 2*B*; similarly, there is no such codon in the corresponding human sequence (see ref. 12)]. The first in-frame (relative to the rest of the RAR-β ORF) AUG is found within the DNA binding domain of RAR-β (Met-113, which is located between the first and second zinc finger of this domain; see ref. 17). However, 10 nucleotides upstream of the beginning of the mRAR-β4 B region, there is an in-frame non-AUG initiator codon, CUG (nucleotide 256 in Fig. 2), which is surrounded by a favorable Kozak sequence (21, 22). Moreover, two short palindromes (underlined in Fig. 1*C*) with the potential of forming stem–loop structures exist immediately downstream of the CUG codon, and such structures have been shown to have the capacity of strongly enhancing the use of both AUG and non-AUG initiators (21–23). We note in this respect that these palindromes, as well as the CUG codon within the same Kozak sequence context, are also present in the corresponding human sequence (Fig. 2*C* and ref. 12).

To test the possibility that initiation of translation could occur from the CUG codon described above, a mRAR-β4 expression vector (mRAR-β4 CTG) was constructed in pSG5 (24) by deleting nucleotides 266–619 in the original mRAR-β2 expression vector mRAR-β0 (17). Similar expression vectors were made in which the CTG codon was mutated to either TTG (mRAR-β4 TTG) or ATG (mRAR-β4 ATG). COS cells were transfected with either one of these three mRAR-β4 expression vectors or with the corresponding expression vectors for mRAR-β1, mRAR-β2, or mRAR-β3 (12). Protein extracts of the transfected cells were analyzed by Western blotting with a rabbit polyclonal antibody preparation specific for a peptide sequence present in the RAR-β F region (18). The mRAR-β4 CTG isoform was synthesized in transfected COS cells, albeit at a lower level than mRAR-β1, -β3, and -β2 isoforms (Fig. 3, compare lane 4 with lanes 1–3). The CTG → ATG mutation (lane 6) resulted in a large increase in mRAR-β4 expression, whereas the TTG mutation (lane 5) abolished its synthesis, thus demonstrating that initiation of translation was indeed taking place at the CUG codon.

Interestingly, a newly discovered species was present in the mRAR-β4 TTG lane with an apparent mass of 37 kDa, which most probably corresponds to initiation of translation at Met-113 (see above). This 37-kDa species was not present in the mRAR-β1, -β2, and -β3 lanes (or in the mRAR-β4 ATG lane), even though a methionine codon is present in all of these isoforms at a position corresponding to Met-113 in mRAR-β4. However, a faint 37-kDa species was seen in the

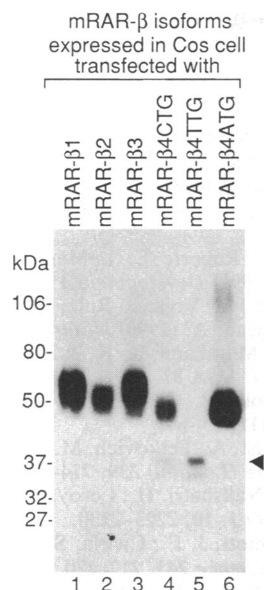

FIG. 3. *In vivo* expression of mRAR-β isoforms. COS cells were transfected with 10 μg of expression vectors for mRAR-β1 (lane 1), mRAR-β2 (lane 2), mRAR-β3 (lane 3), mRAR-β4 CTG (lane 4), mRAR-β4 TTG (lane 5), and mRAR-β4 ATG (lane 6) using the calcium phosphate method (see ref. 17). Lysates from transfected cells were analyzed by SDS/PAGE and immunoblotting (25) with monoclonal antibodies directed against the F region of mRAR-β (kind gift from C. Rochette-Egly and M. P. Gaub, this laboratory). Position of the truncated form of mRAR-β (see text) is indicated by an arrowhead. Size markers are given in kDa.

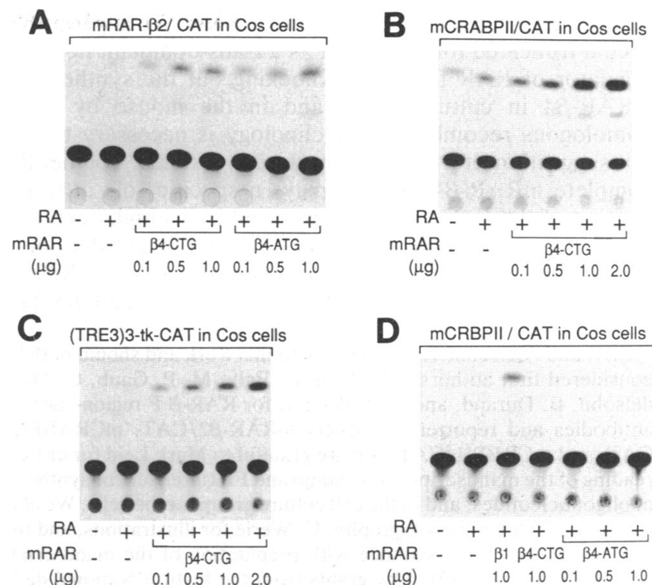

FIG. 4. Transcriptional responsiveness of natural and synthetic RA-responsive promoters to mRAR-β4. The CAT activity resulting in COS cells (see ref. 17) from activation of the reporter genes, mRAR-β2/CAT (10 μg; *A*), mCRABPII/CAT (10 μg; *B*), (TRE3)3-tk-CAT (2 μg; *C*), and mCRBPII/CAT (10 μg; *D*) by cotransfection of increasing concentrations (as indicated) of mRAR-β4 CTG or mRAR-β4 ATG expression vector in the presence of RA (1 μM). (*D*) One microgram of mRAR-β1 expression vector (12) was also cotransfected (as indicated) along with mCRBPII/CAT in COS cells as a positive control.

mRAR-β4 CTG lane. Therefore, a mRAR-β4 isoform truncated for all amino acid sequences located N-terminal to the second zinc finger may possibly be synthesized from mRAR-β4 mRNA, in addition to the CUG-initiated mRAR-β4 isoform whose A region (A4) is only 4 amino acids long (see Fig. 1*C*).

The possibility that the alternative internal initiation of translation in mRAR-β4 mRNA described above may be developmentally regulated is particularly attractive, since the corresponding truncated mRAR-β4 isoform may act as a negative trans-dominant regulator unable to bind to DNA but retaining the capability to form heterodimers with the retinoid X receptors (26). We note in this respect that similar truncated RAR-α isoforms lacking all sequences located N-terminal to the second zinc finger have been previously identified (9) and that a first zinc finger-truncated isoform has also been characterized in the case of the *Drosophila* ecdysone-induced E75 protein, which is also a member of the nuclear receptor superfamily (27). Such truncated forms unable to efficiently bind to DNA response element may be equivalent to the Id molecules, which interact in a dominant negative fashion with basic helix–loop–helix transcriptional regulatory factors (28, 29).

Transcriptional Activation by mRAR-β4. The transcriptional activation function of mRAR-β4 was tested by performing experiments in which COS cells were transfected with the mRAR-β4 CTG or ATG expression vectors, together with reporter genes containing either the synthetic responsive element (TRE3)3 inserted upstream of the thymidine kinase (tk) promoter [(TRE3)3-tk-CAT; see ref. 17] (CAT, chloramphenicol acetyltransferase) or natural RA-responsive promoters, such as those of the RAR-β2 gene (mRAR-β2/CAT; see ref. 18), the *mCRABPII* gene (mouse cellular retinoic acid binding protein II) (mCRABPII/CAT; B. Durand and P.C., unpublished results) and the *mCRBPII* gene (mouse cellular retinol binding protein II) (mCRBPII/CAT; H. Nakshatri and P.C., unpublished results) (Fig. 4; data not shown). RA-dependent stimulation of transcription was observed when mRAR-β4 CTG or ATG was cotransfected with either (TRE3)3-tk-CAT, mRAR-β2/CAT, or mCRABPII/CAT (Fig. 4 *A–C*), but not with mCRBPII/CAT under conditions where transcriptional activation was seen by cotransfecting mRAR-β1, -β2, or -β3 expression vectors (Fig. 4*D*; data not shown). The above observations indicate that a transcriptional activation function is present in mRAR-β4, as previously found for the other RAR-β isoforms (ref. 17; S.N. and P.C., unpublished results). Moreover, the present results indicate that mRAR-β4, whose A region is almost nonexistent, may lack an additional transcriptional modulating activity that is necessary for stimulation of transcription from the mCRBPII promoter and is present in the A region of the other mRAR-β isoforms. Thus, as previously shown in the case of the A/B region of the estrogen and progesterone receptors (7, 8, 30), the A region of RAR-β isoforms may contain a promoter context-specific transcriptional activity.

CONCLUSION

The present data support our previous suggestion that the multiplicity of RAR isoforms may account at least in part for the highly pleiotropic effects of RA in vertebrate development and homeostasis (see refs. 1 and 2 for reviews). In this respect, the promoter context-dependent activation of transcription by the RAR-β4 isoform is particularly noteworthy. Initiation of translation from mRAR-β4 mRNA occurs at a non-AUG leucine initiator codon, thus increasing the list of cases in which initiation from non-AUG codons is used for generating additional regulatory proteins (displaying different localization in some instances; see ref. 31) from a single gene (see refs. 21–23 and references therein). However, initiation from this non-AUG codon appears to be relatively inefficient, which results in alternative usage of an internal methionine codon, thus yielding a mRAR-β isoform truncated for all amino acid sequences located N-terminal of the

second finger of the DNA binding domain. We suggest that such a truncated form may act as a trans-dominant negative regulator of RAR function. Knocking out the synthesis of mRAR-β4 in cultured cells and in the mouse by using homologous recombination technology is necessary to test this suggestion. It will also allow us to investigate whether the complete mRAR-β4 isoform plays a specific role in transduction of the RA signal, as suggested by its high degree of evolutionary conservation and its differential distribution in various tissues when compared with the other mRAR-β isoforms, mRAR-β2 (this study and ref. 12), -β1, and -β3 (12).

S.N. and A.Z. contributed equally to this work and should both be considered first authors. We thank C. Egly, M. P. Gaub, C. Mendelsohn, B. Durand, and H. Nakshatri for RAR-β F region-specific antibodies and reporter constructs mRAR-β2/CAT, mCRABPII/CAT, and mCRBPII/CAT. We are grateful to Mark Leid for critical reading of the manuscript, to A. Staub and F. Ruffenach for synthesis of oligonucleotides, and to the cell culture group for the cells. We also thank B. Boulay for photography, C. Werlé for illustrations, and the secretarial staff for assistance with preparation of the manuscript. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Association pour la Recherche sur le Cancer, and the Fondation pour la Recherche Médicale. S.N. is a recipient of an Institut National de la Santé et de la Recherche Médicale fellowship.

1. Chambon, P., Zelent, A., Petkovich, M., Mendelsohn, C., Leroy, P., Krust, A., Kastner, P. & Brand, N. (1991) in *Retinoids: 10 Years On*, ed. Saurat, J.-H. (Karger, Basel), pp. 10–27.
2. Ruberte, E., Kastner, P., Dollé, P., Krust, A., Leroy, P., Mendelsohn, C., Zelent, A. & Chambon, P. (1991) *Semin. Dev. Biol.* **2**, 153–159.
3. Green, S. & Chambon, P. (1988) *Trends Genet.* **4**, 309–314.
4. Beato, M. (1989) *Cell* **56**, 335–344.
5. Evans, R. (1988) *Science* **240**, 889–895.
6. Gronemeyer, H. (1991) *Annu. Rev. Genet.* **25**, 89–123.
7. Tasset, D., Tora, L., Fromental, C., Scheer, E. & Chambon, P. (1990) *Cell* **62**, 1177–1187.
8. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. & Chambon, P. (1989) *Cell* **59**, 477–487.
9. Leroy, P., Krust, A., Zelent, A., Mendelsohn, C., Garnier, J. M., Kastner, P., Dierich, A. & Chambon, P. (1991) *EMBO J.* **10**, 59–69.
10. Krust, A., Kastner, P., Petkovich, M., Zelent, A. & Chambon, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5310–5314.
11. Kastner, P., Krust, A., Mendelsohn, C., Garnier, J. M., Zelent, A., Leroy, P., Staub, A. & Chambon, P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2700–2704.
12. Zelent, A., Mendelsohn, C., Kastner, P., Garnier, J. M., Ruffenach, F., Leroy, P. & Chambon, P. (1991) *EMBO J.* **10**, 71–81.
13. Mendelsohn, C., Ruberte, E., LeMeur, M., Morriss-Kay, G. & Chambon, P. (1991) *Development* **113**, 723–734.
14. de Thé, H., del Mar Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H. & Dejean, A. (1990) *Nature (London)* **343**, 177–180.
15. Sucov, H. M., Murakami, K. K. & Evans, R. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5392–5396.
16. Ruberte, E., Dollé, P., Chambon, P. & Morriss-Kay, G. (1991) *Development* **111**, 45–60.
17. Zelent, A., Krust, A., Petkovich, M., Kastner, P. & Chambon, P. (1989) *Nature (London)* **339**, 714–717.
18. Smith, W. C., Nakshatri, H., Leroy, P., Rees, J. & Chambon, P. (1991) *EMBO J.* **10**, 2223–2230.
19. Loh, E. Y., Elliott, J. F., Cwirla, S., Lanier, L. L. & Davis, M. M. (1989) *Science* **243**, 217–220.
20. Smith, C. W., Patton, J. G. & Ginard-Nadal, B. (1989) *Annu. Rev. Genet.* **23**, 527–577.
21. Kozak, M. (1991) *J. Biol. Chem.* **266**, 19867–19870.
22. Kozak, M. (1991) *J. Cell Biol.* **115**, 887–903.
23. Xiao, J. H., Davidson, I., Matthes, H., Garnier, J. M. & Chambon, P. (1991) *Cell* **65**, 551–568.
24. Green, S., Issemann, I. & Scheer, E. (1988) *Nucleic Acids Res.* **16**, 369.
25. Rochette-Egly, C., Lutz, Y., Saunders, M., Scheurer, I., Gaub, M. P. & Chambon, P. (1991) *J. Cell Biol.* **115**, 535–545.
26. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J. M., Mader, S. & Chambon, P. (1992) *Cell* **68**, 377–395.
27. Segraves, W. A. & Hogness, D. S. (1990) *Genes Dev.* **4**, 204–219.
28. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61**, 49–59.
29. Sun, X. H., Copeland, N. G., Jenking, N. A. & Baltimore, D. (1991) *Mol. Cell. Biol.* **11**, 5603–5611.
30. Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M.-P. & Chambon, P. (1988) *Nature (London)* **333**, 185–188.
31. Acland, P., Dixon, M., Peters, G. & Dickson, C. (1990) *Nature (London)* **343**, 662–665.