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Murine isoforms of retinoic acid receptor γ with specific patterns of expression

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ABSTRACT **We have characterized seven murine retinoic acid receptor γ cDNA isoforms (mRAR-γ1 to -γ7) generated by alternative splicing of at least seven exons. These isoforms differ from one another in their 5′ untranslated region and in two cases (mRAR-γ1 and -γ2) differ in their N-terminal A region, which is known to be important for differential transactivation by other nuclear receptors. mRAR-γ1 and -γ2, the predominant isoforms, are differentially expressed in adult tissues and during embryogenesis. Most notably, skin contains almost exclusively mRAR-γ1 transcripts. The conservation of the RAR-γ isoforms from mouse to human together with their patterns of expression suggests that they perform specific functions, which may account for the pleiotropic effect of retinoic acid in embryogenesis and development.**

Retinoic acid (RA) may be a natural morphogen conferring positional information through a concentration gradient (see refs. 1–4 for reviews). In mammals, RA is a powerful teratogen whose administration during pregnancy results in a spectrum of craniofacial and limb malformations (5–8) and defects in brain development (refs. 5 and 9; see also ref. 10). RA also has marked effects on differentiation and maintenance of epithelial cells, skin being a major tissue for retinoids (refs. 10–12 and references therein).

The discovery in mouse and human of three nuclear RA receptors (designated RAR-α, -β, and -γ) (13–18) acting as RA-inducible enhancer factors (refs. 19–21 and references therein) provided a basis for understanding how RA signals could be transduced at the level of gene expression. A fourth RAR, δ, possibly corresponding to an additional RAR gene, has been identified in newt (22). Alignment of RARs with other members of the steroid/thyroid hormone nuclear receptor superfamily enabled us to define six distinct regions designated A–F (13, 18) (see Fig. 1 *g* and *h*). Regions C and E, which are involved in DNA and ligand binding, respectively, and region B are highly conserved among all RARs. In contrast, regions A, D, and F are not conserved or are less conserved. However, a comparison of human RARs (hRARs) with murine RARs (mRARs) has shown that the A, D, and F regions of a given RAR subtype are conserved. This evolutionary conservation and the differential distribution of the three RAR mRNAs in mouse tissues strongly suggest that each RAR plays a specific role during development and in the adult animal (10, 11, 17). *In situ* hybridization studies have shown that all three RAR mRNAs exhibit a unique spatiotemporal distribution during mouse development (refs. 10 and 11; unpublished results). More specifically, the pattern of expression of the RAR-γ gene suggests that RAR-γ plays an important role during morphogenesis, chondrogenesis, and differentiation of squamous epithelia (10, 11, 17, 18).

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We previously reported the isolation of several human RAR-γ cDNA species differing from one another in their 5′ sequences located upstream from the B region, possibly generated by differential splicing (18). We report here the characterization of several such murine isoforms.†

MATERIALS AND METHODS

Anchored polymerase chain reaction (PCR) amplification starting from RNA was performed (23) using cDNA synthesized from 1 μg of poly(A)$^+$ RNA and primed with the mRAR-γ C-region antisense oligonucleotide 5′-CCATAGTGGTAGCC-3′. Nucleotides were removed by Sephadex G-50 filtration and the cDNA was oligo(dG)-tailed (24). Two rounds of 20–30 PCR cycles were carried out using successively two appropriate mRAR-γ 3′ primers and the same 5′ anchor primers as in ref. 23. Prior to the second round of amplification, molecules larger than 200 base pairs were excised from a 2% agarose gel, purified by Geneclean (Bio 101), and 1/10th of the material was further amplified using the second 3′ primer, which carried either *Bam*HI, *Hin*dIII, and/or *Xba* I restriction sites. Following directional cloning between one of these sites and the *Not* I site of the pBluescript SK(+) vector, the DNA from positive colonies was sequenced. Anchored PCR on genomic DNA was performed as above, except that the first-strand synthesis was performed on 1 μg of denatured genomic DNA [1 unit of *Thermus aquaticus* (*Taq*) DNA polymerase, 5 min at 55°C]. mRAR-γ-specific primer sequences used for PCR are available upon request.

Untreated and RA-treated (48 hr) F9 embryonal carcinoma cell cDNA libraries were constructed from poly(A)$^+$ RNA in the λZAPII vector (Stratagene). Clones hybridizing under stringent conditions with mRAR-γ cDNA were sequenced using the universal primer, the reverse primer, and the mRAR-γ C-region antisense primer. Other techniques were as described (17, 18, 25).

RESULTS

Cloning of Alternatively Spliced mRAR-γ cDNAs. By using the cloned mRAR-γ cDNA (17) as a probe, six clones (F9.1 to F9.6) were isolated from two λZAPII cDNA libraries of untreated or RA-treated F9 embryonal carcinoma cells. Positive clones fell into three categories (mRAR-γ1, -γ2, and -γ3) according to the sequence upstream from the splice junction separating the exons encoding the A and B regions (A/B junction). mRAR-γ1 sequence (clones F9.1 and F9.2; Fig. 1*a*) was colinear with that of the previous mRAR-γ (17) but had

Abbreviations: RA, retinoic acid; RAR, RA receptor; hRAR, human RAR; mRAR, murine RAR; ORF, open reading frame; PCR, polymerase chain reaction; UTR, untranslated region.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M32068–M32074).

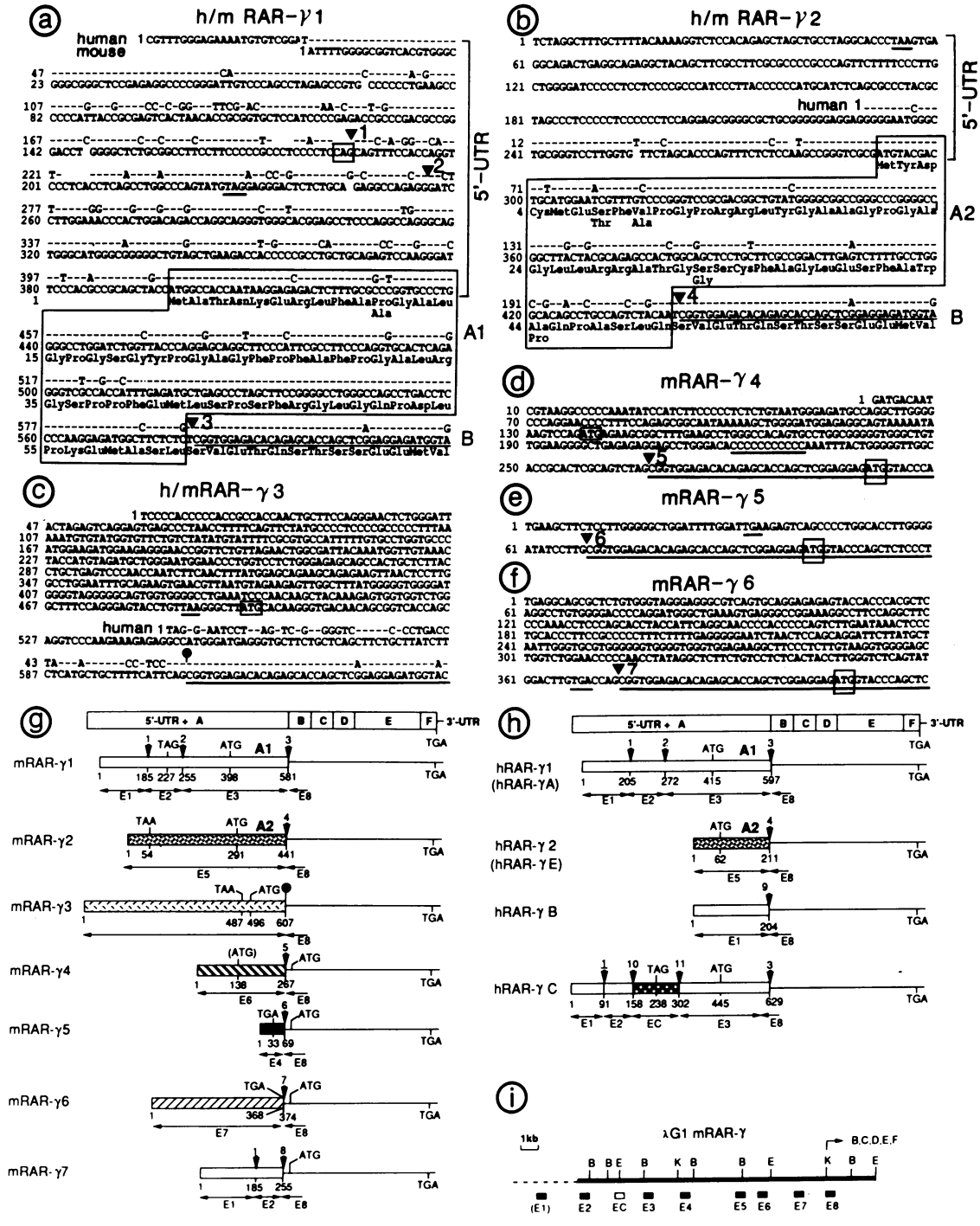

FIG. 1. mRAR-γ isoforms. (*a*) Alignment of the 5′ sequences of mouse (m) and human (h, top lines) RAR-γ1 cDNAs. Gaps have been introduced for optimal alignment, bases differing in hRAR-γ are given, and conserved bases are indicated by dashes. The single amino acid difference in the A1 region (Ala-11 in hRAR-γ1; Pro-11 in mRAR-γ1) is indicated. The 5′ untranslated region (UTR) is indicated, region A1 is boxed, and an in-frame stop codon and the beginning of the B region are underlined. The CAG insertion (see text) is boxed. Filled triangles indicate exon boundaries 1–3 as deduced from sequences of λG1mRAR-γ (sec. *i*). (*b*) Alignment of the 5′ sequences of mRAR-γ2 and hRAR-γ2 cDNAs. (*c*) The 5′ sequence of mRAR-γ3 cDNA. An ATG codon in frame with the common mRAR-γ open reading frame (ORF) is boxed; the preceding in-frame stop codon is underlined. Human genomic sequence upstream of the B region of hRAR-γ gene has been determined by anchored PCR and aligned with the mouse sequence (given on top; same representation as in *a* and *b*). ▼, The 5′ limit of the exon encoding region B. (*d*) The 5′ sequence of mRAR-γ4 cDNA (see text). The stretch of 11 cytosines is underlined. An ATG codon in frame with the common RAR-γ ORF is boxed. (*e* and *f*) The 5′ sequences of mRAR-γ5 and -γ6 cDNAs, respectively. (*g*) mRAR-γ isoforms. The general organization of mRAR-γs (17, 18) is depicted at the top and mRAR-γ1 to -γ7, as deduced from sequence data (*a–f*), are schematized below. The ORF common to all isoforms is indicated by a line spanning regions B–F. Arrows indicate boundaries between exons (E1–E8). (*h*) hRAR-γ isoforms, represented as in *g*. Clones hRAR-γB and hRAR-γC are described in ref. 18. Splicing boundaries (arrowheads) are inferred from our knowledge of the structural organization of mRAR-γ isoforms. The additional exon present in hRAR-γC is called EC. (*i*) The 5′ region of the mRAR-γ gene. Genomic clone λG1mRAR-γ was obtained by screening an L-cell genomic library with a 5′ mRAR-γ1 probe (data not shown). *Bam*HI (B), *Eco*RI (E), and *Kpn* I (K) restriction sites and the approximate location of the exons (*g*) are indicated. Location of the putative mouse EC exon is also indicated. Exon E1 (in parentheses) has not been mapped. With the exception of E6 (see text), the exonic sequences were identical in mRAR-γ cDNA isoforms and λG1mRAR-γ. kb, Kilobase.

150 additional 5′ nucleotides and a CAG triplet inserted at position 183 (boxed in Fig. 1*a*). mRAR-γ1 shared extensive homology with hRAR-γA (ref. 18; designated hereafter hRAR-γ1), including the 5′ UTR (Fig. 1*a*). A similar CAG insertion exists at the corresponding position of the hRAR-γF cDNA clone isolated from a T-47D breast cancer cell cDNA library and otherwise colinear with hRAR-γ1 (unpublished results). Southern blot analysis and DNA sequencing of a λ genomic DNA clone encoding the 5′ region of the mRAR-γ gene (λG1mRARγ, Fig. 1*i*, to be published elsewhere) indicated that the 5′ UTR and A region (A1, Fig. 1*a*) of mRAR-γ1 are encoded in a minimum of three exons (E1–E3; see Fig. 1 *a* and *g* for the locations of exon junctions 1, 2, and 3; note that E1 may correspond to more than one exon).

The mRAR-γ2 cDNA (clones F9.3 to F9.5) possessed sequences upstream of the A/B junction that were different from those of mRAR-γ1. These and additional 5′ sequences obtained by anchored PCR revealed the presence of an ATG in frame with the common mRAR-γ ORF. Furthermore, the F9.5 clone possessed this common ORF in its entirety, thus indicating that mRAR-γ2 cDNA encodes a putative mRAR-γ2 protein and differs from mRAR-γ1 mRNA only in its A region (A2 in Fig. 1*b*) and 5′ UTR. These two regions are encoded in exon E5, which is also present in λG1mRAR-γ (Fig. 1*i*; see Fig. 1 *b* and *g*). The mRAR-γ2 sequence immediately upstream of the A/B junction was similar to that of the hRAR-γE cDNA clone (ref. 18; designated hereafter hRAR-γ2). Additional sequencing of the 5′ UTR and A region of hRAR-γ2 (using clones obtained by anchored PCR on RNA from human fetal skin) revealed that they were both highly homologous to their mouse counterparts (Fig. 1*b*).

The mRAR-γ3 sequence upstream of the A/B junction (Fig. 1*c*) was established using the F9.6 clone and two clones (11.5d/1 and 11.5d/2) isolated from an 11.5-day embryo cDNA library in λgt10 (17). The 11.5d/1 clone possessed the ORF encoding mRAR-γ regions B–F. Since the mRAR-γ3 sequence contains an upstream ATG (boxed in Fig. 1*c*) in frame with the common mRAR-γ ORF, the 11.5d/1 clone may correspond to an additional mRAR-γ protein with an alternative A region. However, sequencing of λG1mRAR-γ upstream from exon E8 (Fig. 1*i*) indicated that the 5′ mRAR-γ3 sequence corresponds to the intronic sequence immediately upstream of E8 (note also a mouse B1 repetitive sequence located between positions 222 and 308). Furthermore, the human genomic sequences upstream of E8 did not reveal any significant conservation (Fig. 1*c*), which suggests that the mRAR-γ3 cDNA clones correspond to partially spliced primary transcripts.

Additional mRAR-γ cDNA clones possessing alternative upstream sequences were obtained by anchored PCR (23) with poly(A)$^+$ RNA isolated from newborn mouse skin, 8.5-, 9.5-, 11.5-, and 14.5-day embryos, and P19 and F9 embryonal carcinoma cells. Sequences corresponding to mRAR-γ1, -γ2, and -γ3 were again found, with mRAR-γ1 and -γ2 accounting for ≈90% of the clones irrespective of the origin of the RNA. Four additional types of clones were obtained (mRAR-γ4 to -γ7) with sequences diverging from those of mRAR-γ1 to -γ3, and from each other, exactly at the A/B splice junction, upstream of exon E8 (Fig. 1).

The original mRAR-γ4 clones were isolated from 8.5-day (one clone) and 9.5-day (one clone) embryo RNA. Further 5′ sequences were obtained by anchored PCR with mRAR-γ4-specific 3′ primers (Fig. 1*d*). A variability in the length of the poly(dC) stretch (9–11 residues; underlined in Fig. 1*d*) was observed. The clone containing 11 cytosine residues has an ATG in frame with the mRAR-γ common ORF (boxes in Fig. 1*d*), thus conceptually encoding an additional mRAR-γ protein with an alternative A region. However, only 7 cytosines were found in exon E6, which contains the corresponding sequence in the genomic DNA clone λG1mRAR-γ. Whether this variability reflects a true mouse polymorphism or corresponds to cloning artifacts is unknown. In any case, mRAR-γ4 in which E6 is spliced to E8 cannot be a partially spliced pre-mRNA (see Fig. 1 *i* and *g*). PCR amplification using poly(A)$^+$ RNA from 9.5-day embryos, a 5′ primer specific for mRAR-γ4, and a 3′ primer specific to the common mRAR-γ 3′ UTR demonstrated the existence of mRAR-γ4 transcripts possessing the entire B–F mRAR-γ ORF (data not shown).

mRAR-γ5 and -γ6 clones (Fig. 1 *e* and *f*) were obtained from RNA of RA-treated F9 cells and from RNA of skin, F9 cells, and P19 cells, respectively. Both isoforms possess an in-frame stop codon upstream of the common mRAR-γ ORF. PCR amplification using RNA from F9 cells, 5′ primers specific for either mRAR-γ5 or mRAR-γ6, and a 3′ primer corresponding to the common mRAR-γ 3′ UTR established the presence of mRAR-γ5 and mRAR-γ6 transcripts colinear with the B–F mRAR-γ ORF. Thus, the mRNAs corresponding to mRAR-γ5 and -γ6 probably encode identical proteins lacking an A region, with a N-terminal methionine corresponding to the first ATG of E8 (Fig. 1*g*). However, they differ in their 5′ UTRs, which are encoded in two separate exons (E4 for mRAR-γ5 and E7 for mRAR-γ6) of λG1mRAR-γ (see Fig. 1 *i* and *g*).

mRAR-γ7 cDNA (Fig. 1*g*) was isolated using 9.5-day embryo RNA. Its sequence upstream of the A/B junction was identical up to its very 5′ end to that of the first 255 bases of mRAR-γ1 encoded in exons E1 and E2 (Fig. 1*g* and data not shown). Thus mRAR-γ7 results from alternative splicing that joins E2 to E8, whereas in mRAR-γ1 E2 is joined to E3. There is no in-frame ATG or stop codon upstream of E8 in the mRAR-γ7 cDNA sequence, which indicates that the corresponding mRNA may encode a mRAR-γ protein lacking an A region. Again PCR amplification carried out as above established the existence of mRAR-γ7 transcripts containing the mRAR-γ B–F ORF (data not shown).

Human Homologs to mRAR-γ cDNA Isoforms. We have noted previously (18) and above the high degree of conservation between mRAR-γ1 and -γ2 and their human counterparts, hRAR-γ1 and -γ2 (hRAR-γA and hRAR-γE in ref. 18). This homology encompasses both the protein-coding region A and the 5′ UTR, indicating that the counterparts of mRAR-γ exons E1, E2, E3, and E5 are well conserved in the human genome and that both regions must be functionally important.

The conservation of mRAR-γ exons E6 and E7, present in mRAR-γ4 and -γ6, respectively, was investigated by comparative Southern blot analysis of human and mouse genomic DNA, using probes specific for these two mouse exons (101–267 and 1–373 for mRAR-γ4 and -γ6, respectively; see Fig. 1 *d* and *f*). In both cases, specific cross-hybridization was observed with restriction digests of human genomic DNA (data not shown).

In addition to the already mentioned hRAR-γA (-γ1), -γE (-γ2), and -γF cDNAs, we have described three T-47D cell hRAR-γ cDNAs with diverging sequences (hRAR-γB, -γC, and -γD; see Fig. 1*h* and ref. 18). Sequence comparisons (18) indicate that hRAR-γB corresponds to an alternative splicing between the human counterparts of mRAR-γ exons E1 and E8. hRAR-γC is identical to hRAR-γ1 but contains an additional sequence inserted between E2 and E3 (Fig. 1 *g* and *h*), which suggests that there could be an additional mouse exon (designated here EC) located between E2 and E3. Finally, the sequence of hRAR-γD (18) diverges from the other RAR-γ sequences at a point that does not correspond to any of the identified mouse splice sites. Furthermore, the diverging sequence of hRAR-γD corresponds to that of hRAR-γ1 at positions 334–352, but in the reverse orientation (18), suggesting that hRAR-γD could be a cloning artifact.

Specific Pattern of Expression of mRAR-γ Isoforms. Probes specific for mRAR-γ1 through -γ6 isoforms were used to

investigate their expression in various adult mouse tissues, embryo stages, and RA-responsive embryonic stem cells and embryonal carcinoma cells (Fig. 2*a*). mRAR-γ1 RNA was readily detected as an ≈3.3-kb transcript on Northern blots from adult lung, embryonal carcinoma and embryonic stem cells, and total embryos at various stages, but the highest expression was observed in adult and newborn skin. The mRAR-γ2-specific probe revealed a 3.1-kb transcript, which was expressed at a much lower level in skin and roughly at similar levels as mRAR-γ1 RNA in lung, embryonal carcinoma and embryonic stem cells, and embryos. A single 6.6-kb transcript was detected in embryonal carcinoma cells (open triangle) with the mRAR-γ6 probe. Whether this transcript corresponds to a mature mRAR-γ6 mRNA or to a partially processed pre-mRNA remains to be seen. The mRAR-γ4 and -γ5 probes did not reveal any hybridizing RNAs in the samples examined, even on overexposed blots (data not shown). In contrast, the mRAR-γ3 probe detected an ≈1.6-kb RNA from adult liver, brain of 16.5-day embryo, and 14.5-day total embryo. This 1.6-kb RNA, which was not detected with a total mRAR-γ cDNA probe (17), is transcribed from the antisense strand (unpublished result). No additional transcript hybridizing with the mRAR-γ3 probe was detected, except for a 6.6-kb RNA in F9 cells, indicating that mRAR-γ3 could correspond to a partially processed RNA (see above). The ≈14.5-kb RNA that was detected with all probes in skin (black triangle) may also represent partially processed transcripts.

Clearly, mRAR-γ1 and -γ2 are the predominant mRAR-γ forms in tissues and embryos that have been analyzed. To examine more quantitatively the relative abundance of mRAR-γ1 and -γ2 transcripts in the various RNA preparations, RNase protection assays were performed with RNA probes spanning the common B region and either the A1 (Fig. 2 *b* and *d*) or the A2 (Fig. 2 *c* and *e*) region. The relative amount of the other RAR-γ RNAs [mRAR-γ(Σ-1) and mRAR-γ(Σ-2) in Fig. 2 *b–e*] was also quantified in each case. The amount of mRAR-γ1 and mRAR-γ2 relative to total mRAR-γ (mRAR-γΣ) is indicated at the bottom of Fig. 2 *d* and *e*. mRAR-γ1 was the predominant RAR-γ isoform in skin (≥90%), whereas mRAR-γ2 was very low (≤5%) in this tissue. In contrast, mRAR-γ2 was the predominant isoform in F9 and embryonic stem cells. Both isoforms were represented in the embryo, but there was a relative decrease in mRAR-γ2 transcripts during the course of embryogenesis, with a concomitant increase of mRAR-γ1 transcripts (compare lanes 8–12 in Fig. 2 *d* and *e*).

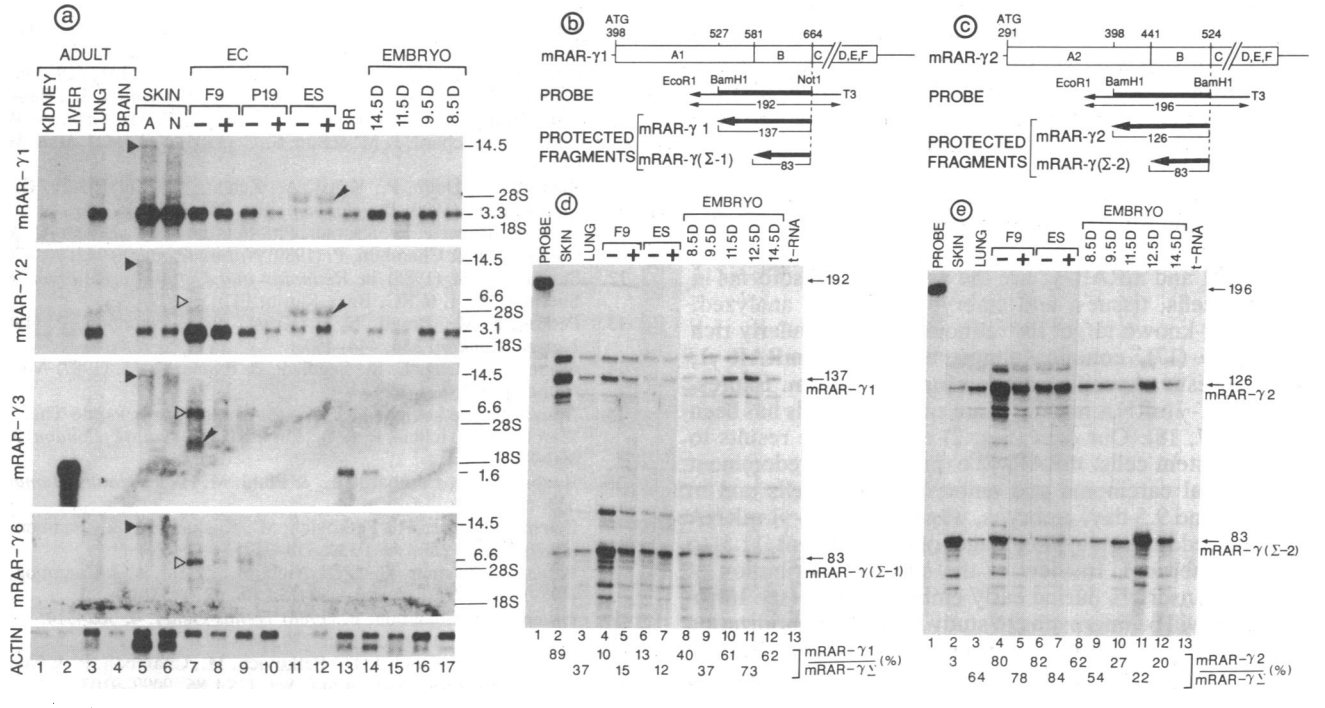

FIG. 2. Distribution of mRAR-γ transcripts. (*a*) Northern blot analysis. Poly(A)$^+$ RNA (4 μg per lane) from adult (A) and newborn (N) tissues, untreated (−) or RA-treated (+) embryonal carcinoma (EC) cells, total embryos collected at various days (D) postcoitum, and 16.5-day embryonic brain (BR) or total RNA (15 μg per lane) from untreated (−) or RA-treated (+) embryonic stem (ES) cells was electrophoresed in a 1% agarose gel containing 1.1 M formaldehyde. Blots were hybridized with probes prepared by PCR (26) and specific for mRAR-γ1, -γ2, -γ3, or -γ6. An actin cDNA probe (17) was used to check the integrity of RNA. Positions of 18S and 28S rRNA and sizes (kb) of mRAR-γ transcripts are shown at right. Open and filled triangles indicate putative splicing intermediate transcripts (see text). Arrowheads in mRAR-γ1 and -γ2 panels indicate nonspecific hybridization with 28S RNA (lanes 11 and 12). Arrowhead in mRAR-γ3 panel points to a remnant of mRAR-γ2 hybridization, due to incomplete removal of the probe. Exposure time was 12 hr (mRAR-γ1 and -γ2; actin) or 60 hr (mRAR-γ3 and -γ6). (*b*) Experimental design for mRAR-γ1 transcript quantification. A fragment from nucleotide 528 (Fig. 1*a*) to the 3′ end of the B region was subcloned in pBluescript SK(+) between *Bam*HI and *Not* I sites, yielding BSK-mRAR-γ1(A/B), which was linearized with *Eco*RI. An antisense RNA probe (192 nucleotides long) was synthesized from the T3 promoter (T3). mRAR-γ1 and transcripts corresponding to the other mRAR-γ isoforms [mRAR-γ(Σ−1)] yielded 137- and 83-nucleotide hybrids, respectively, following RNase digestion (25). (*c*) Experimental design for mRAR-γ2 transcript quantification. A fragment from nucleotide 399 (Fig. 1*b*) to the 3′ end of the B region was subcloned into the *Bam*HI site of pBluescript SK(+), yielding BSK-mRAR-γ2(A/B), which was linearized with *Eco*RI. An antisense RNA probe (196 nucleotides long) was synthesized with T3 polymerase. mRAR-γ2 and transcripts corresponding to the other mRAR-γ isoforms [mRAR-γ(Σ−2)] yielded 126- and 83-nucleotide hybrids, respectively. (*d* and *e*) RNase protection assays for mRAR-γ1 and -γ2 transcripts were performed with mRAR-γ1-specific (*d*) or mRAR-γ2-specific (*e*) probes (see above and lane 1), using either 1 μg of poly(A)$^+$ RNA from various sources [symbols as in *a*], 10 μg of total RNA from untreated (−) or RA-treated (+) ES cells, or 5 μg of tRNA (lane 13). Exposure time was 3 hr (lane 1), 48 hr (lanes 2–7), or 120 hr (lanes 8–13). Relative values given at the bottom are percentages calculated from densitometric scanning of the bands corresponding to the protected fragments and indicate the amount of mRAR-γ1 (*d*) or mRAR-γ2 (*e*) transcripts relative to total RAR-γ transcripts. Scanning values have been standardized with respect to the number of labeled cytidine residues in each protected fragment. Error is estimated to be within 5–10%.

DISCUSSION

We report the isolation and characterization of several mouse RAR-γ cDNA isoforms, all differing in their sequences upstream from the point corresponding exactly to the 5′ end of exon E8, which encodes the B region (Fig. 1 *g–i*; arrowheads 3 and 4–7 in Fig. 1 *a–f*). In contrast, the previously reported mRAR-γ regions B–F (17) appear to be common to all of these isoforms. These differing 5′ UTR and A regions are generated by alternative splicing of at least 7 exons (E1–E7 in Fig. 1), which span at least 14.2 kb (the distance between E2 and E8). It is unknown whether all of these exons are present in the same primary transcript or whether alternative promoters are operating in the mRAR-γ locus. mRAR-γ1 (mRAR-γ in ref. 17) and mRAR-γ2 mRNAs encode two mRAR-γ proteins that differ in their A regions (Fig. 1) and 5′ UTRs. Moreover, both mRAR-γ1 mRNA (17) and mRAR-γ2 mRNA (unpublished results) encode receptors that act as RA-inducible transcriptional enhancer factors. mRAR-γ5, -γ6, and -γ7 (and possibly mRAR-γ4) mRNAs all appear to encode the same putative mRAR-γ protein, which lacks region A and the first 11 amino acids of region B (Fig. 1). However, mRAR-γ4 to -γ7 mRNAs differ in their 5′ UTRs, which are encoded in different exons.

That the RAR-γ isoforms may be functionally important is indicated by their evolutionary conservation. hRAR-γ1 and hRAR-γ2 are strikingly similar to their mouse counterparts (≥90%) in the 5′ UTR and A region (Fig. 1 *a*, *b*, and *h*). Furthermore, sequences cross-hybridizing with mRAR-γ exons E6 and E7 are present in the human genome, indicating the existence of human counterparts to mRAR-γ4 and -γ6. The previous finding of human isoforms hRAR-γB and -γC (see Fig. 1*h* and ref. 18) suggests that at least two additional mRAR-γ isoforms remain to be discovered.

mRAR-γ1 and mRAR-γ2 are the predominant isoforms in all mouse cells, tissues, and embryos that were analyzed. Skin, a well-known target for retinoids and particularly rich in mRAR-γ (17), contains almost exclusively mRAR-γ1, which suggests a specific function for this isoform. Expression of RAR-γ mRNA in embryonal carcinoma cells has been reported (17, 18). Our data (Fig. 2) extend these results to embryonic stem cells. mRAR-γ2 expression is predominant in embryonal carcinoma and embryonic stem cells and in early (8.5- and 9.5-day) embryos, whereas mRAR-γ1 mRNA becomes predominant at later stages of development (11.5- to 14.5-day embryos). In view of the specific distribution of mRAR-γ transcripts during early embryogenesis (see Introduction), it will be interesting to study by *in situ* hybridization the distribution of RAR-γ isoforms.

The evolutionary conservation of RAR-γ isoforms and their differential pattern of expression indicate that they probably perform specific functions. Functional analysis of the estrogen and progesterone receptors has shown that their N-terminal regions contain transcriptional activation functions that can exhibit cell and target-gene promoter specificity (19, 27–29). Thus mRAR-γ1 and -γ2 (and possibly -γ4), which contain distinct A regions, may control the transcription of specific subsets of target genes in different cell types. Similarly, mRAR-γ5 and -γ6, which lack an A region, may regulate the expression of other target genes. Finally, the variability in the 5′ UTRs may play a role in the stability and/or translational efficiency of the mRAR-γ mRNAs (see refs. 30 and 31 for regulatory functions of 5′ UTRs). These 5′ UTRs have the potential to form stable secondary structures (data not shown).

The complexity of the organization and expression of the RAR-γ gene that is emerging from this study is reminiscent of that of other key developmental genes (refs. 32 and 33 and references therein). This complexity probably serves the same purpose in all cases, (*i*) generating several regulatory factors from a single gene by using alternative splicing and multiple promoters and (*ii*) allowing the regulation of the stability and/or translation efficiency of the corresponding mRNAs by producing different 5′ UTRs. Since multiple isoforms have also been found for the other two mRARs, α and β (unpublished results), we begin to see how a single ligand such as RA can be so pleiotropic in its effects and play a crucial role in embryogenesis and development.

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