The chicken retinoid-X-receptor- γ gene gives rise to two distinct species of mRNA with different patterns of expression

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Retinoids are metabolites of vitamin A that can regulate gene expression in a range of embryonic and adult cell types. They do this by binding to nuclear receptors belonging to the steroid/thyroid hormone receptor superfamily of ligand-activated transcription factors. Vertebrates possess two classes of nuclear retinoid-receptor genes, each with three members. These are the RAR- α , RAR- β and RAR- γ genes and the RXR- α , RXR- β and RXR- γ genes. In this paper we show by cDNA cloning and ribonuclease protection that the chicken RXR- γ gene gives rise to two mRNA species (RXR- γ 1 and RXR- γ 2) that differ at their 5' ends. The two mRNAs have different tissue distributions in the 10-day-old chick embryo. RXR- γ 2 mRNA was present in the eye and dorsal root ganglia but was undetectable in the liver. In contrast, RXR- γ 1 mRNA was present in liver, was undetectable

in dorsal root ganglia and was just detectable in the eye, where it was much less abundant than RXR- γ 2 mRNA. The predicted protein products of the RXR- γ 1 and RXR- γ 2 mRNAs differ at their N-termini, in a region thought to modulate transcriptional transactivation by the receptor. These results show that at least one of the retinoid-X-receptor (RXR) genes gives rise to more than one protein product, a principle previously established for the retinoic acid-receptor (RAR) genes. The existence of multiple RXR protein isoforms would increase the range of heterodimers formed between RXRs and other nuclear receptors, including RARs and the receptors for thyroid hormone, vitamin D and peroxisome proliferators. This could increase the diversity of transcriptional responses mediated by these molecules.

INTRODUCTION

A number of naturally occurring metabolites of retinol (vitamin A), including all-*trans*-retinoic acid, 9-*cis*-retinoic acid and 3,4-didehydro-retinoic acid, are believed to function as signalling molecules that regulate cell behaviour during embryonic development and in adult life. This activity is thought to account for the teratogenic effects of retinoid excess in humans [1] and other mammals [2], and for the efficacy of retinoids as drugs for the treatment of some chronic dermatoses and certain forms of cancer [3,4]. Retinoids exert their effects on cells by means of a complex signal-transduction system. At the heart of this system is a family of nuclear retinoid receptors that belong to the steroid/thyroid-hormone nuclear receptor superfamily. These proteins have a modular structure of six domains, designated A to F. Binding of retinoic acid to the E domain converts the receptor into an active transcription factor that can regulate transcription from promoters containing a retinoic acid response element (RARE). Dimerized receptors bind to RAREs via their C domains. Two classes of nuclear retinoid receptor have been identified in vertebrates. These are the retinoic acid receptors (RARs) and the retinoid-X receptors (RXRs). There are three RAR genes, termed RAR- α , RAR- β and RAR- γ [5–9], and three RXR genes, termed RXR- α , RXR- β and RXR- γ [10–14]. The DNA-binding C domain is highly conserved between the RAR and RXR classes, but there is no significant amino acid identity between their other domains. As a consequence of the differences between the ligand-binding E domains of the RAR and RXR classes of receptor, they have different retinoid-binding

specificities. In particular, RARs bind and respond to both all-*trans*-retinoic acid and 9-*cis*-retinoic acid, while RXRs bind and respond only to 9-*cis*-retinoic acid [15,16].

In order to bind to RAREs with high affinity, RARs must form heterodimers with RXRs [12,14,17,18]. Such heterodimers bind to RAREs in the absence of ligand and activate transcription in its presence. RXRs also form heterodimers with thyroid-hormone receptor, vitamin D receptor and peroxisome proliferator-activated receptor, increasing both the affinity of DNA binding and the efficiency of ligand-dependent transcriptional transactivation from promoters containing cognate response elements for these receptors [12,14,17–19]. While RARs require RXRs in order to function, the RXRs can function independently. Thus in the presence of 9-*cis*-retinoic acid, RXRs form active homodimers [20].

Analysis of RAR cDNA clones indicates that the RAR- α , RAR- β and RAR- γ genes each encode more than one protein as a result of alternative promoter usage and/or alternative splicing [21]. In this paper we show that the chicken RXR- γ gene gives rise to two distinct mRNAs, which have different patterns of expression in the embryo. The two mRNAs contain different first coding exons and their predicted protein products differ at their N-termini.

MATERIALS AND METHODS

RNA isolation

Fertilized chicken eggs were obtained from Poyndon Farm, Waltham Cross, Herts., U.K. and were incubated for 10 days at

Abbreviations used: RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element.

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The nucleotide sequence data reported will appear in EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X58997.

Figure 1 Origin of probes used in ribonuclease protection assays and sizes of the expected protected fragments

RXR- γ sequences are shown as boxes. Sequences shared by pR2 and pR1 are open. Sequences unique to pR2 or pR1 are hatched. The pBluescript SK⁺ polylinker is shown as a single thin line. The fragments that should be protected by the two species of RXR- γ mRNA are shown as thick lines beneath the constructs. Abbreviations: E, *EcoRI* site; S, *SmaI* site; T7, site of transcription initiation for T7 RNA polymerase; T3, site of transcription initiation for T3 RNA polymerase. Lengths of fragments are shown in nucleotides.

38 ± 1 °C. Tissue was dissected into sterile PBS and total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method [22].

Northern-blot analysis

Poly(A)⁺-enriched RNA (2 μ g per track) was fractionated on 1% (w/v) agarose-Mops-formaldehyde gels and blotted on to GeneScreen Plus membrane (NEN, Stevenage, Herts., U.K.). Membranes were hybridized with a ³²P-labelled RNA probe specific for chicken RXR- γ gene transcripts as previously described [13].

Ribonuclease protection assays

³²P-labelled RNA probes were prepared as previously described [13], using the templates shown in Figure 1. Ribonuclease protection assays were performed using RNase ONE (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions. Briefly, 2.5×10^6 c.p.m. of probe and 25 μ g of total RNA were mixed, precipitated in ethanol and resuspended in 30 μ l of

hybridization buffer [80% (v/v) deionized formamide, 40 mM Pipes (pH 6.4), 0.4 M NaCl, 1 mM EDTA]. Samples were incubated at 85 °C for 5 min and then at 45 °C overnight. Digestion buffer [300 μ l; 10 mM Tris/HCl (pH 7.5), 0.2 M NaCl, 5 mM EDTA] containing 3–4 units of RNase ONE was then added to each sample. After incubation at 37 °C for 1 h, 20 μ g of yeast poly(A)⁺ RNA and 5 μ l of 10% (w/v) SDS were added. Samples were precipitated in ethanol, resuspended in 4 μ l of sequencing-gel loading buffer and analysed on 6% (w/v) polyacrylamide-urea sequencing gels. Sizes of protected fragments were estimated by reference to DNA sequence ladders run in adjacent tracks.

Screening of cDNA and genomic libraries

The adult chicken liver library, constructed in λ ZAP, was purchased from Stratagene (La Jolla, CA, U.S.A.). We have described the construction previously, in λ EMBL3, of the chicken genomic library [23]. Libraries were plated at high density and screened as previously described [24] using DNA probes labelled with [α -³²P]dCTP (specific radioactivity 800 Ci/mmol; NEN, Stevenage, Herts., U.K.) by the method of Feinberg and Vogelstein [25]. The probe used to screen the cDNA libraries was a 118 bp *SmaI* fragment corresponding to nucleotides 221–339 of the chicken RXR- γ cDNA clone pR2 [13]. The probe used to screen the genomic library was the whole 1713 bp *EcoRI* insert of the chicken RXR- γ cDNA clone pR2 [13].

Nucleotide sequencing

Dideoxy-sequencing was performed on denatured double-stranded DNA templates using Sequenase 2.0 (USB, Cleveland, OH, U.S.A.) according to the manufacturer's instructions. Oligonucleotide primers were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. Nucleotide sequences were analysed using the PC-Genie software package (Intelligenetics, Mountain View, CA, U.S.A.).

Southern-blot analysis

Restriction digests were electrophoresed on 0.8% (w/v) agarose gels and blotted on to GeneScreen Plus membrane (NEN, Stevenage, Herts., U.K.). Membranes were hybridized with ³²P-labelled DNA probes, washed and autoradiographed as described previously [23]. DNA probes were labelled as described above.

RESULTS

Isolation of two distinct species of chicken RXR- γ cDNA clones

We have previously reported the isolation of a chicken RXR- γ cDNA clone, pR2 [13]. We showed, by *in situ* hybridization to sections of chick embryos, that RXR- γ transcripts were present in the liver, in migrating neural-crest cells, in some neural-crest-derived tissues including cranial ganglia, dorsal root ganglia, sympathetic ganglia and spinal nerves [13], and in the developing retina (E. A. P. Seleiro and P. M. Brickell, unpublished work). In order to characterize these RXR- γ transcripts further we probed Northern blots of poly(A)⁺-enriched RNA isolated from different tissues of 10-day-old chick embryos. As shown in Figure 2, a single RNA species of approx. 2.2 kb was detectable in RNA isolated from dorsal root ganglia (lane 3) and the eye (lane 4). In contrast, the liver contained a rather diffuse band of RNAs, ranging in size from approx. 2.0 to 2.2 kb (Figure 2, lane 5).

We supposed that the existing RXR- γ cDNA clone, pR2, which was isolated from a 10-day-old chick embryo cDNA

Figure 2 Northern blot of poly(A)⁺-enriched RNA (2 μ g/lane) isolated from 10-day-old chick embryo head (lane 1), body (lane 2), dorsal root ganglia (lane 3), eye (lane 4) and liver (lane 5)

The blot was probed with a ³²P-labelled antisense RNA probe specific for chicken RXR- γ transcripts. This probe corresponds to nucleotides 786–1713 of cDNA clone pR2 [13].

library, was derived from mRNA present in the retina, as *in situ* hybridization to sections of 10-day-old embryos (results not shown) suggested that the retina is the major source of RXR- γ RNA in the 10-day-old embryo, largely as a result of the great size of the eye in relation to the rest of the embryo. We therefore screened a chicken liver cDNA library for RXR- γ cDNA clones, in order to determine why RXR- γ mRNA in embryonic liver differed in size from that in embryonic eye (Figure 2). We isolated and sequenced a number of clones and found that they had nucleotide sequences identical to that of pR2 along most of their length [13], but differed from pR2 at their 5' ends. The longest of these clones was pR1 (Figure 3a). None of the clones isolated from the liver cDNA library contained the 5' sequences found in pR2. The sequences of the unique 5' regions of pR1 and pR2 (Figure 3a) are shown in Figure 3(b), along with the amino acid sequences of the predicted protein products of the corresponding mRNAs in this region. The predicted protein products of the two mRNAs differ only in their N-terminal sequences.

Analysis of chicken RXR- γ mRNAs by ribonuclease protection

To determine whether pR1 and pR2 represent true mRNAs (RXR- γ 1 mRNA and RXR- γ 2 mRNA respectively) and whether these are tissue-specific, we performed ribonuclease protection experiments. Templates for probe synthesis were prepared by subcloning the 5' *Eco*RI–*Sma*I fragment of each clone into pBluescript SK⁺, as indicated in Figure 1. Radiolabelled antisense RNA probes were prepared with T3 RNA polymerase, using *Eco*RI-linearized pR2-ES or pR1-ES as templates, and were hybridized to total RNA isolated from tissues of 10-day-old chick embryos. Figures 4 and 5 show the fragments of the two probes that were protected from subsequent ribonuclease digestion.

The pR2-ES probe was 274 nucleotides long (Figure 1; Figure 4, lane 1). Total RNA from 10-day-old chick embryo liver protected a 114-nucleotide fragment (Figure 4, lane 6), indicating that this tissue contains RXR- γ 1 mRNA (Figure 1). No 218-nucleotide fragment was detectable, indicating that 10-day-old chick embryo liver contains no detectable RXR- γ 2 mRNA.

Figure 3 (a) Structures of cDNA clones isolated from a 10-day-old chick embryo cDNA library (pR2) and from a chicken liver cDNA library (pR1) and (b) nucleotide sequences of the unique 5' regions of pR2 and pR1 [shaded in (a)] together with the amino acid sequences of the predicted protein products of these regions

(a) Regions in which the nucleotide sequences of pR1 and pR2 are identical are shown as open boxes. Regions in which the nucleotide sequences of the two clones differ are shaded. Clone pR1 also has 26 nucleotides at its 3' end that are absent from pR2. The positions of the *Eco*RI linker sites (E) that flank the inserts are shown, as are the positions of the *Sma*I sites (S) referred to in the text. Nucleotides are numbered from the 5' end of each insert. We have published the nucleotide sequence of the region indicated by the open boxes previously [13].

Total RNA from 10-day-old chick embryo eye (Figure 4, lane 7) and 10-day-old chick embryo dorsal root ganglia (Figure 4, lane 8) protected fragments of 218 and 198 nucleotides. In addition, a low-abundance 114-nucleotide fragment was present in Figure 4 (lane 7). The presence of the 218-nucleotide fragment indicates that 10-day-old chick embryo eye and dorsal root ganglia contain RXR- γ 2 mRNA (Figure 1). The presence of the weak 114-nucleotide signal in Figure 4 (lane 7) indicates that 10-day-old chick embryo eye also contains RXR- γ 1 mRNA (Figure 1), but at much lower abundance than RXR- γ 2 mRNA. Whole 10-day-old chick embryo RNA (Figure 4, lane 5) protected a 218-nucleotide fragment, corresponding to RXR- γ 2 mRNA, as well as the 198-nucleotide fragment. No 114-nucleotide fragment was visible in this lane, presumably because RXR- γ 1 mRNA constitutes only a very small proportion of the mRNA population in the whole embryo. For the same reason, we were never able to isolate pR1-like cDNA clones from whole 10-day-old chick embryo cDNA libraries, despite exhaustive screening of two independent libraries. The 218-, 198- and 114-nucleotide fragments were not protected by yeast total RNA (Figure 4, lane 4).

The 198-nucleotide fragment seen in Figure 4, lanes 5, 7 and 8, results from artefactual cleavage of perfectly matched hybrids and does not represent protection by another mRNA species. The evidence for this is shown in Figure 4, lane 3. Here, we hybridized the radiolabelled antisense-strand RXR- γ 2 RNA probe to unlabelled sense-strand RXR- γ 2 RNA. The probe and

Figure 4 Analysis of RXR- γ mRNAs by ribonuclease protection

A 32 P-labelled probe was synthesized with T3 RNA polymerase, using *Eco*RI-linearized pR2-ES as a template. Lane 1 contains undigested probe. Lanes 2 and 3 contain probe fragments protected by hybridization to unlabelled mRNA synthesized with T7 RNA polymerase, using *Sma*I-linearized pR1-ES (lane 2) or *Sma*I-linearized pR2-ES (lane 3) as templates. Lanes 4–8 contain probe fragments protected by hybridization to 40 μ g of total RNA from yeast (lane 4), whole 10-day-old chick embryo (lane 5), 10-day-old chick embryo liver (lane 6), 10-day-old chick embryo eye (lane 7) and 10-day-old chick embryo dorsal root ganglia (lane 8). The probe and protected fragments expected are shown in Figure 1. The sizes of protected fragments are indicated in nucleotides.

the unlabelled RNA were both synthesized *in vitro*, using the same template. In addition to the expected 221-nucleotide fragment (Figure 1: 218 nucleotides shared between pR2-ES and RXR- γ 2 mRNA, plus the six nucleotides of the *Eco*RI site, minus three nucleotides of the *Sma*I site), there was a protected fragment of 198 nucleotides. Since the probe and synthetic unlabelled RNA were perfectly matched, this 198-nucleotide fragment must have arisen by artefactual cleavage at a site where the perfectly matched duplex was unstable.

The pR1-ES probe was 253 nucleotides long (Figure 1; Figure 5, lane 1). Total RNA from 10-day-old chick embryo liver protected a 197-nucleotide fragment (Figure 5, lane 6), confirming that this tissue contains RXR- γ 1 mRNA (Figure 1). No 114-nucleotide fragment was detectable, confirming that 10-day-old chick embryo liver contains no detectable RXR- γ 2 mRNA. Total RNA from 10-day-old chick embryo eye (Figure 5, lane 7) and 10-day-old chick embryo dorsal root ganglia (Figure 5, lane 8) protected a 114-nucleotide fragment, although this is barely visible in Figure 5, lane 8. The presence of the 114-nucleotide fragment confirms that 10-day-old chick embryo eye and dorsal root ganglia contain RXR- γ 2 mRNA (Figure 1). RXR- γ 1

Figure 5 Analysis of RXR- γ mRNAs by ribonuclease protection

A 32 P-labelled probe was synthesized with T3 RNA polymerase, using *Eco*RI-linearized pR1-ES as a template. Lane 1 contains undigested probe. Lanes 2 and 3 contain probe fragments protected by hybridization to unlabelled mRNA synthesized with T7 RNA polymerase, using *Sma*I-linearized pR1-ES (lane 2) or *Sma*I-linearized pR2-ES (lane 3) as templates. Lanes 4–8 contain probe fragments protected by hybridization to 40 μ g of total RNA from yeast (lane 4), whole 10-day-old chick embryo (lane 5), 10-day-old chick embryo liver (lane 6), 10-day-old chick embryo eye (lane 7) and 10-day-old chick embryo dorsal root ganglia (lane 8). The probe and protected fragments expected are shown in Figure 1. The sizes of protected fragments are indicated in nucleotides.

mRNA, which should protect a 197-nucleotide fragment, was not detectable in 10-day-old chick embryo eye or dorsal root ganglia using this probe. As would be expected, whole 10-day-old chick embryo RNA (Figure 5, lane 5) protected fragments of 197 and 114 nucleotides. Neither fragment was protected by yeast total RNA (Figure 5, lane 4).

No other protected fragments were detectable with either probe, indicating that there are no other detectable RXR- γ mRNA species that diverge from the RXR- γ 1 and RXR- γ 2 mRNAs upstream of the *Sma*I site shown in Figure 3(a).

Taken together, these data show that RXR- γ 2 mRNA is present in 10-day-old chick embryo eye and dorsal root ganglia, but undetectable in liver. In contrast, RXR- γ 1 mRNA is present in 10-day-old chick embryo liver and is just detectable in the eye, where it is much less abundant than RXR- γ 2 mRNA.

Genomic organization of the unique 5' exons of chicken RXR- γ 1 and RXR- γ 2 mRNAs

To determine the genomic organization of the unique regions found at the 5' ends of pR1 and pR2, we screened a λ EMBL3 chicken genomic library using the radiolabelled insert of cDNA clone pR2 as a probe. The resulting genomic clone, λ RXRG1,

Figure 6 Southern blots of λ RXRG1 DNA digested with *EcoRI* (E), *PstI* (P) or *SmaI* (S) and probed with the radiolabelled *EcoRI*–*SmaI* inserts of pR1-ES (a) or pR2-ES (b)

The approximate sizes of the hybridizing fragments are shown in kilobase pairs.

Figure 7 Map of the insert of λ RXRG1 showing the genomic organization of the alternative 5' exons found in chicken RXR- γ mRNAs

The nucleotide sequences of the ends of the exons (upper-case letters) and of the flanking genomic DNA (lower-case letters) are shown. The positions of the *PstI* fragments RP1 and RP2 are indicated. The insert contains other *PstI* fragments, but these have not been mapped. Abbreviations: E, *EcoRI*; P, *PstI*; S, *SmaI*.

hybridized to the radiolabelled *EcoRI*–*SmaI* inserts of both pR1-ES and pR2-ES (Figure 6), but failed to hybridize to a series of radiolabelled oligonucleotide probes corresponding to sequences shared by cDNA clones pR1 and pR2 (data not shown), including a probe corresponding to the 5' end of the shared region (nucleotides 84–103 of pR1, nucleotides 105–124 of pR2). These data indicated that the insert contained the sequences unique to pR1 and pR2, but none of the shared sequences.

Restriction analysis of λ RXRG1 DNA showed that the insert was approx. 15 kb long. It contained a single *SmaI* fragment of approx. 6 kb, flanked by terminal fragments of approx. 1.7 kb and 7.3 kb (Figure 7). The pR1-ES probe hybridized to the 6 kb *SmaI* fragment (Figure 6), indicating that this fragment contained the sequences unique to pR1 (Figure 7). The pR1-ES probe also hybridized to a *PstI* fragment of approx. 1.4 kb (Figure 6). This

fragment, named RP1, was sub-cloned and sequenced. It was 1370 bp long and contained a *SmaI* site, which was located 18 bp from one end. The 83-nucleotide sequence unique to pR1 appeared as a single exon, beginning 592 bp downstream of the *SmaI* site. These data enable the pR1 exon to be mapped at the position shown in Figure 7. The pR1 exon was flanked at its 3' end by a consensus splice donor sequence (Figure 7). No consensus splice acceptor sequence was found upstream.

Restriction analysis of λ RXRG1 DNA showed that the insert contained a single *EcoRI* fragment of approx. 2.5 kb, flanked by terminal fragments of approx. 12 kb and 0.5 kb (Figure 7). The pR2-ES probe hybridized to the 2.5 kb *EcoRI* fragment (Figure 6), indicating that this fragment contained the sequences unique to pR2 (Figure 7). The pR2-ES probe also hybridized to a *PstI* fragment of approx. 1.1 kb (Figure 6). This fragment, named RP2, was sub-cloned and sequenced. It was 1137 bp long and contained an *EcoRI* site, which was located 330 bp from one end. The 104-nucleotide sequence unique to pR2 appeared as a single exon, beginning 206 bp downstream of the *EcoRI* site. These data enable the pR2 exon to be mapped at the position shown in Figure 7. The pR2 exon was flanked at its 3' end by a consensus splice donor sequence (Figure 7). No consensus splice acceptor sequence was found upstream.

The two exons were separated by approx. 9.7 kb of genomic DNA (Figure 7).

DISCUSSION

In this paper we have shown that the chicken RXR- γ gene gives rise to at least two mRNA species (RXR- γ 1 and RXR- γ 2) with distinct 5' exons that are spliced on to common downstream sequences. We have shown previously that the chicken RXR- γ gene is expressed in the developing retina and liver and in migrating neural-crest cells and some of their derivatives, including peripheral nervous tissue [13]. We have shown here that the tissue distributions of the RXR- γ 1 and RXR- γ 2 mRNAs in the 10-day-old chick embryo differ from each other. RXR- γ 2 mRNA was found in the eye, was detectable with the pR2-ES probe in dorsal root ganglia (Figure 4, lane 8), but was undetectable in the liver. RXR- γ 1 mRNA was found in the liver, it was just detectable with the pR2-ES probe in the eye (where it was much less abundant than RXR- γ 2 mRNA) and was undetectable in dorsal root ganglia (Figure 4, lane 8). The cells of the dorsal root ganglia are derived from the neural crest, and it seems likely that the RXR- γ transcripts in migrating neural-crest cells and other neural-crest derivatives are also predominantly of the RXR- γ 2 class. It is, of course, possible that liver contains very low levels of RXR- γ 2 mRNA that are below the limit of detection. Similarly, dorsal root ganglia could contain very low levels of RXR- γ 2 mRNA that are below the limit of detection.

The finding that liver contains RXR- γ 1 mRNA but no detectable RXR- γ 2 mRNA does not explain why a diffuse band of mRNAs was detected when a Northern blot of polyadenylated liver RNA was probed for RXR- γ transcripts (Figure 2, lane 5). Our preliminary data suggest that this diffuse band may result from the existence of additional, rare, liver-specific RXR- γ mRNA species. It cannot result from cross-hybridization to RAR mRNAs, as the probe shares no significant sequence identity with these [13]. Neither can it result from cross-hybridization to RXR- α mRNA, as this is much longer than 2 kb (E. A. P. Seleiro and P. M. Brickell, unpublished work). It could be the result of cross-hybridization to RXR- β mRNA, although an RXR- β gene has not yet been identified in chickens.

One possible interpretation of the structures of the two chicken RXR- γ mRNAs and the genomic organization of the two 5'

exons is that the two mRNAs arise as a result of alternative promoter use, with the $\gamma 1$ promoter being active primarily in the liver, and the $\gamma 2$ promoter being active primarily in the eye and dorsal root ganglia. However, we have not shown that the two cDNA clones are full-length, and we have not identified transcriptional start sites or tissue-specific promoter activity in the regions upstream of the two alternative 5' exons. It therefore remains a possibility that the RXR- $\gamma 1$ and RXR- $\gamma 2$ mRNAs share a common upstream exon that we have not cloned and that they are both transcribed from the same promoter. In this case, their tissue-specific distribution would have to result from tissue-specific differences in splicing patterns.

The predicted protein products of the RXR- $\gamma 1$ and RXR- $\gamma 2$ mRNAs differ in their N-terminal sequences. By comparison with other members of the steroid/thyroid-hormone-receptor superfamily, this region corresponds to the A domain. Nagpal et al. [25a] have shown that the N-terminal A/B domains of RARs and RXRs contain a region that modulates the transcriptional activation of promoters containing RAREs, in a manner that is dependent upon promoter context and cell type. It is therefore possible that the chicken RXR- $\gamma 1$ and RXR- $\gamma 2$ proteins will have different transcription activation characteristics. It may be that the two proteins regulate different or overlapping sets of target genes in the different tissues in which they are expressed.

Our data show that the chicken RXR- γ gene resembles the RAR genes, each of which gives rise to more than one mRNA species, with different or overlapping tissue distributions. The mouse RAR- α gene encodes two major protein isoforms ($\alpha 1$ and $\alpha 2$), which differ in their A domains. These proteins are encoded by two mRNAs that arise as a result of transcription from two promoters [26,27]. The mouse RAR- β gene encodes four major protein isoforms ($\beta 1$, $\beta 2$, $\beta 3$ and $\beta 4$), which also differ in their A domains. These are encoded by four mRNAs generated by alternative splicing of primary transcripts from two promoters [28,29]. The mouse RAR- γ gene encodes two major protein isoforms ($\gamma 1$ and $\gamma 2$), which also differ in their A domains [30,31]. These are encoded by mRNAs generated by alternative splicing of one primary mRAR- γ transcript. The human RAR genes appear to encode a similar range of proteins [7]. Recently, Li and Linney [32] have shown that the mouse RXR- γ gene also gives rise to at least two mRNAs, with the potential to encode two different proteins. One of these has a very similar sequence to that encoded by chicken RXR- $\gamma 2$ mRNA, as previously reported [11,13]. The other is a truncated receptor that lacks an A domain. The two mouse RXR- γ mRNAs also show tissue-specific expression, but these do not correlate with the distribution of the two chicken RXR- γ mRNAs, since the avian and rodent RXR- γ genes have quite different expression patterns ([11,13]; E. A. P. Seleiro, A. Rowe, P. Georgiades and P. M. Brickell, unpublished work).

RXR proteins can function both as homodimers [20] and as heterodimers with RARs, thyroid-hormone receptor, vitamin D receptor and peroxisome proliferator-activated receptor [12,14,17–19]. The existence of multiple RXR protein isoforms increases the range of potential RXR–RXR homodimers and RXR-containing heterodimers that can form. If homodimers and heterodimers involving different RXR protein isoforms regulate different sets of target genes, this would greatly increase the diversity of transcriptional responses mediated by these nuclear receptors.

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