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Peroxisome proliferator-activated receptor γ1 (PPAR-γ1) as a major PPAR in a tissue in which estrogen induces peroxisome proliferation

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Abstract Estradiol administration induces peroxisome proliferation and the production of 3-hydroxy fatty acid pheromones in the uropygial glands of the duck, but not in the goose gland, which does not produce such pheromones. We isolated a peroxisome proliferator-activated receptor (PPAR)γ1 cDNA from a duck uropygial gland cDNA library. Northern blots revealed two transcripts, PPARγ1 and γ2, and showed that PPARγ was expressed at higher levels than PPARα in the uropygial gland of the duck. Although PPARγ2 was expressed in both duck and goose uropygial gland, PPARγ1 was expressed only in the duck gland, which responds to estrogen by peroxisome proliferation. In NIH 3T3 transfected cells, PPARγ1 was activated by peroxisome proliferators such as Wy-14 643, clofibric acid and Ly-171 883 causing induction of the target marker gene. By cotransfection with a plasmid containing α-*cis*-retinoic acid receptor RXRα, the induction increased up to 9-fold. These results suggest that PPARγ1 may be involved in peroxisome proliferation while PPARγ2 may be involved in lipid metabolism.

Key words: Peroxisome proliferator-activated receptor γ; Uropygial gland; cDNA cloning; Peroxisome proliferator; Transfection

1. Introduction

In rodents peroxisome proliferation is induced by a group of structurally diverse chemicals called peroxisome proliferators [1]. Peroxisomes produce reactive oxygen and hydrogen peroxide that can cause oxidative damage to DNA that may lead to carcinogenesis [2]. A group of nuclear hormone receptors called peroxisome proliferator-activated receptors mediate the transcriptional activation of the genes that encode enzymes involved in peroxisomal fatty acid β-oxidation. To date at least three isoforms of PPAR, α, β and γ, have been found in amphibians, rodents and humans [3–11]. Targeted disruption of the gene that encodes the α form in mice eliminated the pleiotropic effects of peroxisome proliferators [12]. Recently PPARγ has received more attention since PPARγ is highly expressed in adipose tissues and is uniquely involved in the conversion of fibroblasts into cells of the adipose lineage [13–16]. Recently it has been shown to be involved in the regulation of inflammatory responses [17,18] and in the formation of foam cells from macrophages in the presence of oxidized low-density lipoprotein (oxLDL) [19–21]. Thus PPARs can play central and diverse roles in lipid metabolism. Alternative splicing of one gene product yields two transcripts of PPARγ, γ1 and γ2 [7,22], and the ratio of the two isoforms varies with both species and tissues [23–27]. In rodents PPARγ2 is the major form synthesized in adipose tissues, suggesting that this form may be involved in adipocyte differentiation [6], and PPARγ1 has been found only as a minor component in both adipose and non-adipose tissues [23,24]. In cattle PPARγ1 was expressed at higher levels than γ2 in spleen, lung, and ovary [25]. In human, while only PPARγ1 was expressed in muscle [26], the ratio of PPARγ2/γ1 in adipose tissues was found to be correlated with the extent of human obesity [27], consistent with the observation that PPARγ2 was responsible for adipocyte differentiation. However, the function of PPARγ1 remains unknown.

A new role for peroxisomes was discovered when it was found that during the mating season the uropygial glands of female mallards, which normally produce wax esters, proliferate peroxisomes to produce sex pheromones, 3-hydroxy fatty acid diesters [28]. That this seasonal switch in lipid synthesis is mediated by estrogens was shown by the observation that peroxisome proliferation and diester production could be induced in the uropygial gland with no detectable changes in the liver by intramuscular administration of estradiol [29]. The high degree of correlation of hepatic peroxisome proliferation with liver cancer observed in rodents [2] raises the possibility that estrogen-induced peroxisome proliferation in the estrogen target tissues could play a similar role in carcinogenesis in such tissues. To date nothing is known about the molecular basis of estrogen-induced peroxisome proliferation and PPARs have not been cloned from any avian tissues.

In this paper, we report the cloning of PPARγ1 cDNA from mallard duck uropygial gland. We show that the γ1 and γ2 isoforms of PPAR are produced at similar levels in the duck uropygial gland, whereas only the γ2 form is expressed in the goose uropygial gland, which does not produce peroxisomes in response to estradiol treatment. The unique occurrence of PPARγ1 as a major form of this receptor in the duck gland implicates it in estradiol-induced peroxisome proliferation. We also show here that in transient transfection the duck PPARγ1 can be activated by peroxisome proliferators such as Wy-14,643, and more potently by prostaglandin J_2 series.

2. Materials and methods

2.1. Chemicals

Wy-14 643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid) was purchased from Chemsyn Science Laboratories (Lenexa, KS, USA). Ly-171 883 and 9-*cis*-retinoic acid were purchased from Biomol Research Laboratories (Plymouth Meeting, PA, USA). The prostaglandins were obtained from either Biomol Research Laboratories or from Cayman Chemical Company (Ann Arbor, MI, USA). Clofibric acid and other chemicals were obtained from Sigma. The chemicals used in transient transfection were dissolved in ethanol or DMSO as

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stock solution and applied to the medium at final concentrations in the range of 0.1–0.2%.

2.2. Animals

1–2 year old mallard ducks (*Anas platyrhynchos*) were purchased from Whistling Wings (Hanover, IL, USA) and maintained in outdoor cages. They were injected daily intramuscularly into the flight muscles with 0.1 ml of either 17β-estradiol benzoate (1 mg dissolved in 0.1 ml of 2.5% ethanol in olive oil) for the experimental group or the ethanol/olive mixture for the control group [29].

2.3. Isolation of duck PPARγ cDNA

Poly(A) mRNA was isolated from the mallard duck uropygial glands with the Fastrack kit, following the instruction of the manufacturer (Invitrogen), using 100 mg tissue per ml of the denaturing buffer. Then 2 μg of mRNA was heated at 70°C for 5 min in the presence of *Not*I primer adapter, and incubated with 5× reverse transcription buffer, RNasin ribonuclease inhibitor, 4.0 mM sodium pyrophosphate, and AMV reverse transcriptase in a volume of 25 μl at 42°C for 2 h (Promega). The second strand synthesis reaction was performed by adding 23 units of *E. coli* DNA polymerase I and 0.8 units of *E. coli* RNase H. The double stranded cDNA was cloned into the *Eco*RI-digested λgt11 vector (Stratagene). Recombinant plaques (0.5 million) of this library were screened with a 180-bp DNA fragment from the DNA binding region of PPAR. This probe was obtained by PCR using the double stranded cDNA of duck uropygial gland as the template, and with two degenerate primers that correspond to the amino acid sequences from the DNA binding region of PPAR. The sequences of the primers were as follows: 5′-GGG GAT CCT (A/C/G/T)TG (C/T)GG (A/C/G/T)GA (C/T)AA (G/A)GC and 5′-GGG AAT TCC (G/T)(A/G)C A(A/C/G/T)T (A/G)(C/T)T G(A/G)C A. Four clones were purified after three sequential rounds of plating and hybridization. Bacteriophage DNA from the selected positive plaques was prepared according to standard methods [30]. cDNA insert fragments were eletroeluted from 1% agarose gel and subcloned into the *Eco*RI site of vector pGEM7Z (Promega) and sequenced with specific primers.

2.4. Isolation of duck PPARα cDNA fragment

About 0.3–1 μg of mRNA from untreated duck tissues was used. The reverse transcription reaction was performed at 42°C for 1 h with superscript II reverse transcriptase. The template RNA was removed by incubation with RNase H at 55°C for 20 min and the resultant DNA was used as template for PCR reactions. Two oligonucleotides for PCR were designed based on the cDNA sequence which is specifically conserved among all of the known PPARαs from human, rodents and *Xenopus laevis*: primer ap5, 5′-AAG AAC TTC AAC ATG AAC; and primer ap6, 5′-CGA TCT CCA CAG CAA AT. The following degenerate oligonucleotide primer specific for PPARα isoform was designed for priming the reverse transcription reaction of the first strand of cDNA from mRNA: primer ap4, 5′-G(G/C)A C(G/A)T G(T/C/G)A C(A/G)AT (A/C/G)C(C/T) CTC. For the PCR reactions 40 cycles were performed, with 1 min at 94°C for denaturing, 1 min at 48°C for annealing and 2 min at 72°C for extension in each cycle. The PCR product was subcloned and at least three clones from each reaction were sequenced to assure that point mutations did not arise during PCR.

2.5. Northern blot analysis

Poly(A)$^+$ mRNA was prepared from estrogen-treated or untreated mallard duck tissues with the Fastrack kit. Each RNA sample (2 μg) was denatured in formamide and formaldehyde at 65°C for 15 min, and resolved on a 1% agarose gel containing 0.66 M formaldehyde. The RNA was transferred to Duralon-UV membrane (Stratagene) with Turboblotter rapid downward transfer system and baked for 2 h at 80°C. The 2.4-kb duck PPARγl *Eco*RI fragment and the 519-bp PCR product of duck PPARα cDNA were labeled with [α-^{32}P]dCTP using Rediprime DNA labeling system (Amersham). The membranes were prehybridized at 42°C for 2 h in a solution containing 6× SSPE, 5× Denhard's reagent, 50% formamide, 0.1% SDS and 100 μg of denatured salmon sperm DNA, then hybridized with the labeled DNA probes at 42°C overnight. The membranes were washed in 2× SSPE and 0.1% SDS at room temperature for 10 min twice and in 0.1× SSPE and 0.1% SDS at 65°C for 15 and 25 min. Following washing the membranes were exposed to X-ray film.

2.6. Primer extension analysis

A synthetic 30-mer oligonucleotide complementary to the duck PPARγl mRNA close to the 5′ end (primer EXT1, GAT CCA CTG GGC TAA TTC CAA AAT TAA TGG) was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The products were precipitated with 2 M sodium acetate and ethanol and resuspended in water. Either 10–20 μg of mRNA or 60–100 μg of total RNA, which was prepared with RNeasy total RNA kit (Qiagen) was incubated in a total volume of 16 μl with labeled primers in 5× reverse transcriptase buffer containing 250 mM Tris-HCl, 375 mM KCl and 15 mM $MgCl_2$. The annealing was done by heating at 85°C for 15 min and cooling on ice. Then DTT, dNTP and BRL Superscript II RNase H$^-$ reverse transcriptase (200 U) were added and the synthesis of the first strand of cDNA was performed at 50°C for 1 h. The reaction was stopped by heating at 70°C for 15 min and RNase A was used to degrade the template RNA. After extraction with phenol/chloroform, synthesized DNA was precipitated by sodium acetate and ethanol in the presence of 25 μg of yeast tRNA. The final product was dissolved in 10 μl of sequencing stop buffer and analyzed on a 6% polyacrymide sequencing gel.

2.7. Plasmids and transient transfection assay

The *Eco*RI fragment of duck PPARγl cDNA was subcloned into the *Eco*RI site of mammalian expression vector pcDNA3 (Invitrogen). Two complementary 39-mer oligonucleotides were synthesized based on the peroxisome proliferator response element, PPRE, from rat fatty acyl-CoA oxidase promoter with *Bgl*II site overhangs: 5′-gat ct G ACT CCC GAA CGT GAC CTT TGT CCT GGT CCC CTa and 5′-gat ctA GGG GAC CAG GAC AAA GGT CAC GTT CGG GAG TCa. They were phosphorylated with T4 polynucleotide kinase and extracted with phenol/chloroform. The products were annealed to double strand by boiling followed by cooling to room temperature, and subcloned into the *Bgl*II site of pGL_2-promoter vector which contains the SV40 promoter upstream of the luciferase gene (Promega). The positive clones identified from colony hybridization were further analyzed by sequencing and the clone with three consecutive copies of PPRE was used in the transfection experiments as the PPRE-containing reporter vector. The mouse α-*cis*-retinoic acid receptor (RXRα) plasmid was kindly provided by Dr. Ronald M. Evans (Salk Institute).

NIH 3T3 cells were maintained in DMEM high glucose with 10% calf serum. Transient transfection was performed using the Lipofect-AMINE reagent (BRL) by following the standard protocol. Briefly, the cells were split and switched to DMEM without phenol red and with calf serum which had been treated with 2% dextran-coated charcoal. On the next day the cells, which were 60–80% confluent in 35-mm plates, were transfected with 200 ng of duck PPARγl, 200 ng of PPRE-containing luciferase reporter, and 1 μg of β-galactosidase expression vector or with 200 ng of mRXRα as specified. pBluescript plasmid was used to make up the total amount of DNA to 2 μg per sample and each assay was done in triplicate. After 5 h the ligands were added together with the serum-containing medium. The medium was replaced 24 h later with the fresh medium containing ligands, and after 48 h the cells were washed with PBS buffer and harvested.

The cell lysates were obtained by 3–4 cycles of freezing and thawing in a solution of 0.1 M potassium phosphate, pH 8.0, 1 mM DTT and 0.05% Triton. Luciferase activity was measured with the Lumat LB 9501 from Berthold, and normalized with β-galactosidase activities that served as the internal control.

3. Results

3.1. Cloning and sequencing of PPARγl cDNA from the duck uropygial gland

A 180-bp cDNA fragment obtained by PCR using degenerate primers and uropygial gland cDNA as template was confirmed to be a PPAR fragment by sequencing. This PPAR cDNA fragment was used as a probe to screen a cDNA library of duck uropygial gland. The nucleotide sequencing of four cDNA clones thus obtained showed that a 2.4-kb clone contained the complete open reading frame, while another clone of 1.2 kb was found to be a fragment

of the 2.4-kb clone. The other two clones, which were about 2.7 kb in size, were found to be identical to each other. The 5′ ends of these were identical to that of the first clone, but their 3′ ends were 352 bp longer than that of the 2.4-kb clone. The nucleotide sequence and the predicted amino acid sequence of the protein encoded by the open reading frame are shown in Fig. 1. The amino acid sequence has an overall 90% identity with human, hamster and mouse PPARγ1. Most of the con-

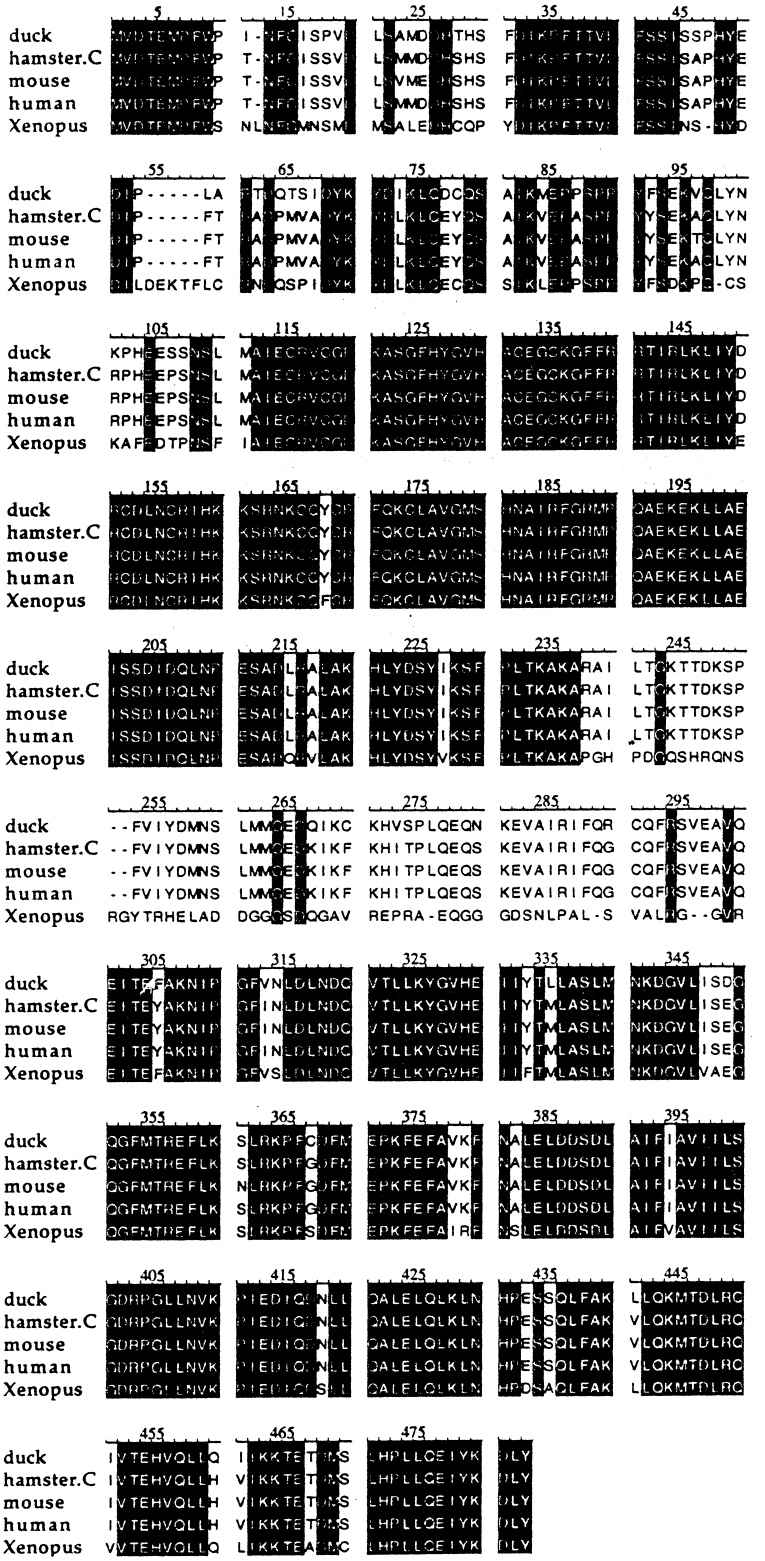

Fig. 1. Alignment of the amino acid sequence of duck PPARγ 1 with other PPARγ proteins. The conserved regions among all of the proteins are shaded. The hamster PPARγ1 was from Chinese hamster CHO cells. Mouse PPARγ1 was from mouse liver. The human PPARγ1 was also from liver.

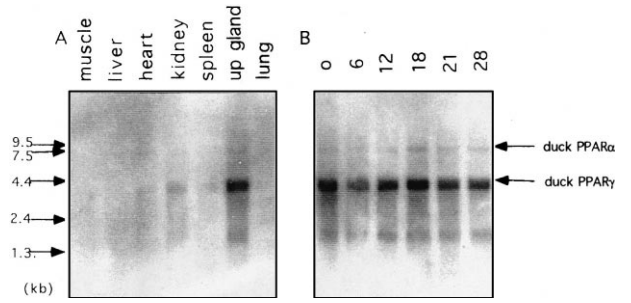

Fig. 2. A: RNA blots showing the tissue distribution of duck PPARα and PPARγ. Two μg of mRNA from each tissue and ^{32}P-labeled probes were used, as described in Section 2. B: RNA blots showing the expression of duck PPARα and PPARγ in the uropygial gland during the treatment of the ducks with 17β-estradiol. Two μg of mRNA from each tissue was applied. The numbers represent the days of 17β-estradiol treatment.

served amino acids were found to be located in the regions of both DNA binding and ligand binding domains.

3.2. PPARα in the uropygial gland of the duck

It is well known that PPARα, the most inducible subtype of receptor, plays a major role in peroxisome proliferation in the rodent liver. We examined its expression in the duck uropygial gland, where estradiol-induced peroxisome proliferation occurs [29]. For this purpose we cloned PPARα from duck by RT-PCR. When RT-PCR was performed with primers specific for the α isoform, RNA from liver, kidney and uropygial gland yielded a 519-bp fragment. The nucleotide sequence of the partial duck PPARα cDNA clone showed 90% homology to PPARα from other sources (data not shown). Northern blot analysis with this DNA fragment as probe showed that PPARα was expressed in heart and uropygial gland at a higher level than in the other tissues, although it was also expressed in kidney and liver (Fig. 2A). When the transcript levels of PPARα and PPARγ were compared on the same blot, the level of expression of the γ isoform was found to be higher than that of the α form in the uropygial gland, suggesting the importance of duck PPARγ in the overall lipid metabolism in the duck uropygial gland. The co-expression of PPARα and γ in the duck uropygial gland probably reflects the extensive and active lipid metabolism in this tissue.

3.3. Expression of PPARγ1 specifically in the duck uropygial gland

Northern blot analysis showed that duck PPARγ was uniquely and highly expressed in the uropygial gland when compared with other tissues. Its expression was also detected in kidney and spleen, but at much lower level (Fig. 2A). PPARγ transcripts were also detected in liver when primer extension analysis was done with high levels of mRNA (Fig. 3). Furthermore, there were two transcripts which were close to each other in size, and both were about 4.4 kb long (Fig. 4). We identify them as duck PPARγ1 and γ2 based on the findings in mouse and human [6,7]. The two transcripts were found to be expressed at similar levels. This finding constitutes the first known example of a tissue where PPARγ1 is a major PPAR. We also examined the expression of PPARγ by Northern blot in the goose uropygial gland, where estrogen does not induce peroxisome proliferation (P.E. Kolattukudy, unpublished data). No PPARγ expression was detected in the liver, but unlike in the duck, only one of the two transcripts, corresponding to PPARγ2, was detected at high levels in the goose uropygial gland. Thus, the occurrence of PPARγ1 as a major PPAR seems to be a unique feature of a tissue that responds to estrogen treatment by peroxisome proliferation.

In order to determine if any isoform of PPARγ was highly expressed exclusively in the duck uropygial gland, primer extension analysis was performed with a 30-mer oligonucleotide which was complementary to the region of 29–59 nucleotide position downstream of the start site of the open reading frame of the duck PPARγ1. Since the 5′ end of the cDNA sequence of the PPARγ2 was not known, other known sequencing reactions were included in parallel to serve as size markers. Three transcripts were detected, two of which were only different by five or six nucleotides in length, while the other was at least 120 bp longer (Fig. 5). The two shorter transcripts were too short to contain the full length of the open reading frame of the γ2 form and therefore are identified as PPARγ1. The results showed that PPARγ1 was highly expressed specifically in the duck uropygial gland while PPARγ2 was highly expressed in both duck and goose uropygial gland.

Since our initial interest was to find the molecular basis for the estrogen-induced peroxisome proliferation in the duck uropygial gland, we examined by Northern blots whether estradiol treatment caused any changes in the relative levels of

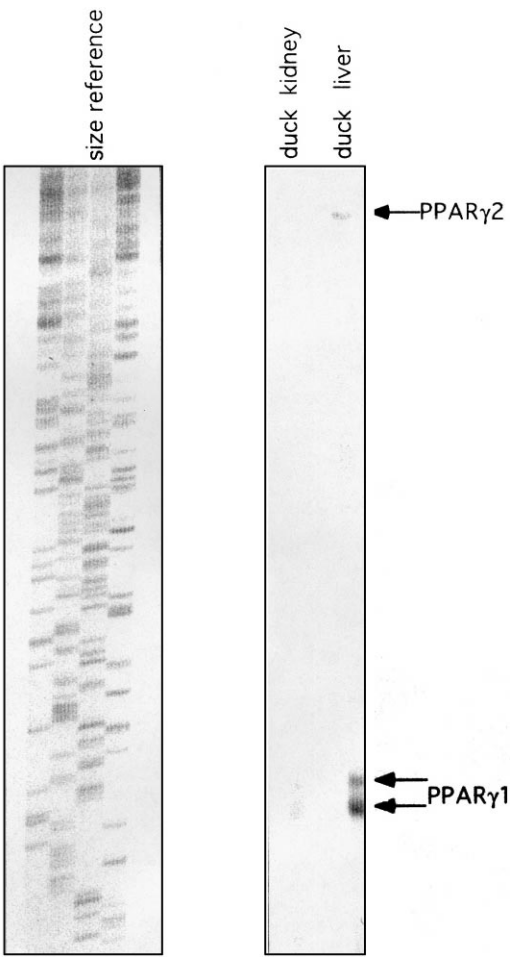

Fig. 3. Primer extension analysis of the PPAR transcripts from duck kidney and liver. Ten μg of duck kidney mRNA and 20 μg of liver mRNA were used.

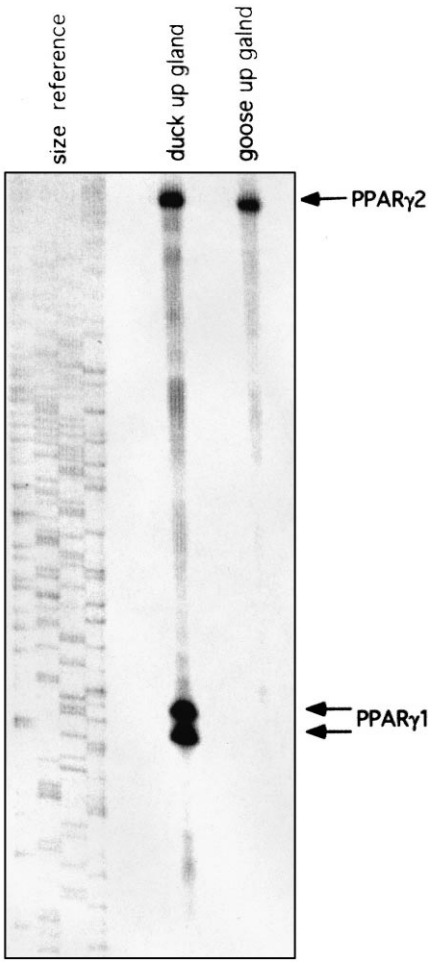

Fig. 4. The expression of PPARγ isoforms in duck and goose uropygial gland and liver as detected by Northern blot. In the goose uropygial gland the top band was found to be at a much lower level than in the duck uropygial gland. Two μg of mRNA from each tissue and ^{32}P-labeled probes were used, as described in Section 2.

PPARγ (Fig. 2B). The uropygial gland secretion samples collected after treatment of the ducks for the various periods of time were analyzed by thin-layer chromatography. In the beginning, the secretion consisted exclusively of short chain esters of fatty alcohols; after 3 days of estradiol treatment, longer chain esters appeared and became the sole product by 9 days of treatment. By 12 days diesters, the characteristic product of peroxisomes, became the dominant component of the secretion demonstrating peroxisome proliferation. The level of expression of PPARγ slightly decreased within the first several days, and then recovered to the original level and remained steady by the time of peroxisome proliferation. Therefore we conclude that estrogen did not induce the expression of PPARγ at the mRNA level. We did not detect any changes in PPARγ1/PPARγ2 ratios resulting from estrogen treatment.

3.4. Activation of duck PPARγ1 by peroxisome proliferators and prostaglandins

To test for the function of the duck PPARγ1, we expressed it in NIH 3T3 cells that are known to have the low background of PPARγ and high level of expression of RXR that would be necessary for the transactivating activity of PPARγ [19]. The cells were also cotransfected with a luciferase reporter plasmid in which three copies of PPRE derived from the rat fatty acyl-CoA oxidase promoter were inserted as enhancer. The transient transfection showed that the duck PPARγ1 was activated by peroxisome proliferators such as 100 μM Wy-14 643 and clofibric acid as well as 33 μM Ly-171 883, and gave rise to up to 3–4-fold induction of the target gene. By cotransfection with mRXRα the maximum induction up to 7–9-fold was observed (Fig. 6). However, when the prostaglandins were tested it was found that prostaglandin D_2 and its metabolites, the prostaglandin J_2 series, were able to induce the target gene expression by 5–6-fold at much lower concentrations (Fig. 7).

4. Discussion

To investigate the mechanisms involved in the induction of peroxisome proliferation by estrogen in its target tissue, the uropygial gland of the duck, we cloned PPAR from this tissue. This is the first PPAR to be cloned from an avian system. The level of expression of the two isoforms of PPARγ was found to be significantly high in duck uropygial gland.

Although it has been known that PPARγ is predominantly expressed in adipose tissue and is considered as the adipocyte determination factor [13,24,31], the actual functions of the two γ isoforms are not clear. The evidence that the PPARγ

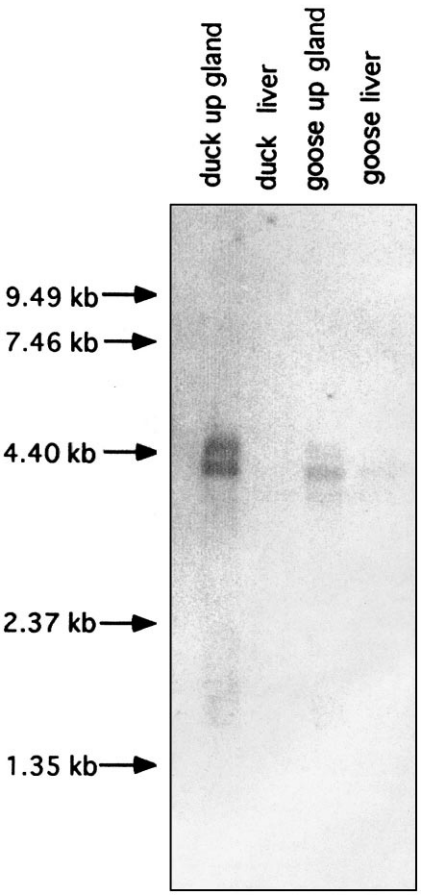

Fig. 5. Primer extension analysis of the PPAR transcripts from goose and duck uropygial glands. Three transcripts were detected in the duck uropygial gland, while two of them were absent in the goose uropygial gland. 100 μg of duck uropygial gland total RNA and 60 μg of goose uropygial gland total RNA were used.

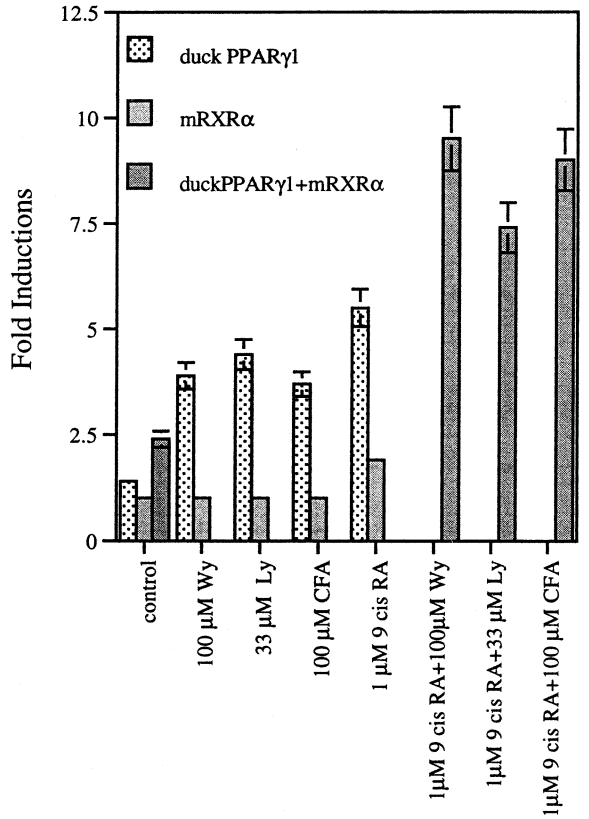

Fig. 6. Activation of duck PPARγ1 by peroxisome proliferators. NIH 3T3 cells were transfected by the duck PPARγ1 expression vector, the mouse RXR expression vector and the PPRE luciferase reporter plasmid. The activators or solvent alone (control) were added to the cell culture medium at the concentrations indicated. The luciferase activities were measured, and the fold inductions were normalized by β-galactosidase activities in the same extracts. Each reaction was done in triplicate. The reference fold induction refers to PPRE luciferase reporter alone in the presence of solvent. Wy: Wy-14 643; Ly: Ly-171 883; RA: *cis*-9-retinoic acid; CFA: clofibric acid.

expressed in rodent adipose tissue is mainly PPARγ2 and the observation that expression of PPARγ2 mRNA was increased in human obesity suggests that PPARγ2 may be responsible for the adipose differentiation [24,28]. However, it has been shown that the N-terminal domain of PPARγ2 is not required for its adipogenic activity [24], and both γ1 and γ2 isoforms can bind to DR-1 (direct repeat with one nucleotide spacing) response elements, which have been identified in the promoters of genes which encode peroxisomal enzymes and of murine-specific adipocyte gene P_2 [32,33]. In addition, thiazolidinediones including BRL-49 653 can activate the two isoforms of human and mouse PPARγ at similar levels [7,34].

Our results show that PPARγ2 is present in both duck and goose uropygial gland, but PPARγ1 is expressed only in the duck. This result further emphasizes the difference between the two PPARγ isoforms. Both duck and goose uropygial glands specialize in the production of large amounts of secretory lipids, just as adipose tissue produces large amounts of storage lipids. On the other hand, estrogen-induced peroxisome proliferation occurs only in the uropygial gland of the duck, but not of the goose. Therefore it is reasonable to suggest that PPARγ1 is involved in estrogen-induced peroxisome proliferation, while PPARγ2 is essential for adipogenesis. Since the two PPARγ isoforms have the ability to transactivate the same target genes and respond to the same group of ligands [24], the difference in their functions is not clear. One possibility is that the additional segment found uniquely in PPARγ2 is involved in the regulating interaction between PPARγ2 and other nuclear receptors, coactivators, or regulators involved in the process of adipogenesis. In fact it is known that a basic leucine zipper transcription factor C/EBP can also bind to and activate the promoters of several adipocyte genes, and the optimal stimulation of adipocyte differentiation is obtained by the combined expression of PPARγ2 and C/EBPα [24,33]. PPARγ was also found to interact with steroid receptor coactivator (ARC-1) and CBP, the protein that binds cAMP response element binding protein (CREB), which is a co-factor of transcription regulation of various genes [35,36]. Recently a 165-kDa protein was reported to be a coactivator of PPARγ and at least the C-terminus of PPARγ was responsible for the interaction [36]. However, the role of the N-terminus of PPARγ was not clear. Another possible regulation might be through phosphorylation, since it has been shown that when activated by PGF_2, MAP kinase (mitogen-activated protein kinase) can block adipogenesis through inhibitory phosphorylation of PPARγ [37]. It is likely that two different forms are used so that different factors can regulate their expressions to meet specific biological functions. Since the uropygial gland is a holocrine gland, it contains cells at different stages of differentiation. It is possible that the two isoforms occur at different levels in the cells

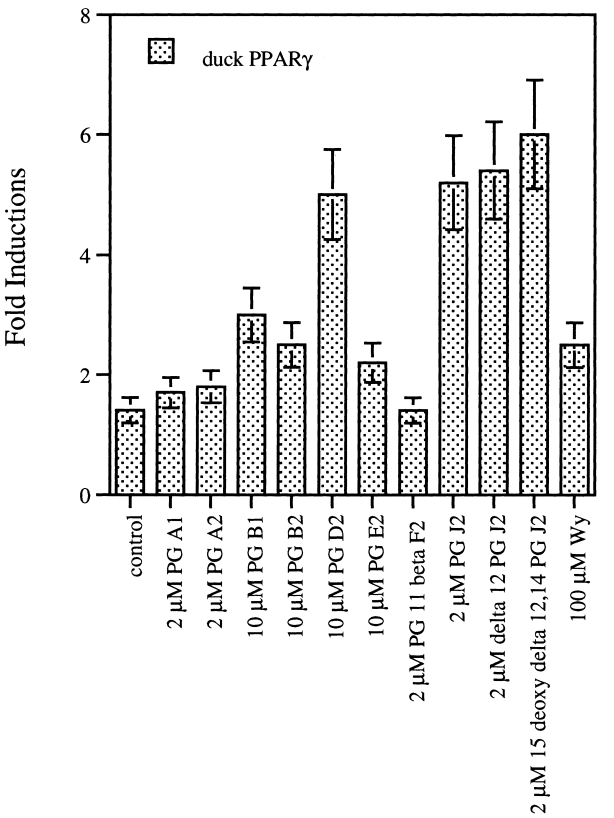

Fig. 7. Activation of duck PPARγ1 by prostaglandins. The transfected NIH 3T3 cells were treated with 10 μM or 2 μM of each of the indicated prostaglandins. Other experimental details are the same as in Fig. 6. Prostaglandin A_2 and prostaglandin J_2 series were toxic to cells at 10 μM.

at different stages of differentiation. This gland has many features of adipocytes, as well as the unique feature of producing the specialized secretory lipids. Thus, the occurrence of PPARγ2, which is found in adipocytes, and the occurrence of PPARγ1 as a major receptor in this gland might imply some functional uniqueness for the two γ isoforms.

Estrogen-induced peroxisome proliferation is a special feature of duck uropygial glands. The unique presence of PPARγ1 as a major PPAR in this tissue implies that this form may be involved in peroxisome proliferation. The microbodies produced by this hormonal induction generate diesters of 3-hydroxy C_8, C_{10} and C_{12} fatty acids [28]. This process may require activation of a slightly different mix of genes from those involved in peroxisome proliferation in the liver, where β-oxidation of fatty acids may be the major function. The induction of the biosynthetic microbodies in the gland may involve different ligands than those that trigger peroxisome proliferation in the liver. Thus, the two processes probably require a unique mix of interacting transcription factors, including the PPARγ1. The molecular partners that interact with PPARγ1 and the mechanisms of their transcriptional activation of the target genes remain to be elucidated. How estrogen causes peroxisome proliferation via PPARγ is not understood. Although estrogen could induce genes regulated by PPAR responsive elements in a cell culture, the degree of induction was thought to be too low to account for the observed peroxisome proliferation and an indirect mechanism was suggested when it was found that estradiol treatment induced the synthesis of a prostaglandin D_2 metabolite that was found to be a potent ligand for PPARγ [38].

Peroxisome proliferation in the liver is associated with tumorigenesis, presumably mediated via the oxidative damage to the DNA caused by the oxidant produced by fatty acid oxidases [2]. The microbodies produced in the uropygial gland as a result of estrogen treatment also produce fatty acid oxidase, and probably cause oxidative damage to DNA. However, since this is a holocrine gland, the cells in which such DNA damage might occur would lyse, emptying the cell contents into the lumen for secretion. Therefore, this hormone-induced peroxisome proliferation would not cause lasting changes such as transformation and tumorigenesis. On the other hand, if estrogen-induced peroxisome proliferation occurs in the target tissues in mammals, tumorigenesis may result. Whether such hormone-induced peroxisome proliferation occurs just prior to tumorigenesis is not known.

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