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Differential Expression of Peroxisome Proliferator-Activated Receptor-α, -β, and -γ during Rat Embryonic Development*

OLIVIER BRAISSANT† AND WALTER WAHLI

Institut de Biologie Animale, Université de Lausanne, 1015 Lausanne, Switzerland

ABSTRACT

The expression patterns of the three different peroxisome proliferator-activated receptor (PPAR) isotypes have been determined during rat embryonic development by *in situ* hybridization. The expression of PPARα starts late in development, with increasing levels in organs such as liver, kidney, intestine, and pancreas, in which it will also be present later in adulthood to regulate its specific target genes. PPARα is also transiently expressed in the embryonic epidermis and central nervous system. PPARγ presents a very restricted pattern of expression, being strongly expressed in brown adipose tissue, in which differentiation it has been shown to participate. Like PPARα, it is also expressed transiently in the central nervous system. Interestingly, PPARα, -β and -γ are coexpressed at high levels in brown adipose tissue. Finally, the high and ubiquitous expression of PPARβ suggests some fundamental role(s) that this receptor might play throughout development. (*Endocrinology* **139:** 2748–2754, 1998)

PEROXISOME proliferator-activated receptors (PPARs) are lipid-activatable transcription factors that belong to the nuclear hormone receptor superfamily (see Ref. 1 for a review). To date, three isotypes of PPARs have been described in amphibians, rodents, and humans: PPARα, PPARβ (also called δ, NUC-1, or FAAR), and PPARγ (2–10). PPARs were shown to be activated by substances that induce peroxisome proliferation (2, 3) as well as by natural fatty acids (4, 11). It is only recently that fatty acids, some eicosanoids, and some hypolipidemic and antidiabetic drugs have been shown to directly bind to PPARs (12–18; see Ref. 19 for a review).

PPAR target genes encode enzymes involved in peroxisomal and mitochondrial β-oxidation, ketone body synthesis, and microsomal ω-hydroxylation, as well as the production of fatty acid binding proteins, apolipoproteins, lipoprotein lipase, malic enzyme, phosphoenolpyruvate carboxykinase, and the brown adipose tissue (BAT) uncoupling protein (see Refs. 1, 20, and 21 for reviews; 22). Thus, PPARs play key roles in different aspects of lipid metabolism and homeostasis. Consistent with the different pathways they regulate, PPARs were shown to be expressed in a wide range of tissues of the adult organism (3, 7, 23–25). We have shown recently, using *in situ* hybridization, that in the adult rat, PPARα is expressed in cells with high catabolic rates of fatty acids and high peroxisome-dependent activities (hepatocytes, cardiomyocytes, proximal tubules of kidney, intestinal mucosa). PPARβ is expressed ubiquitously and is abundant in most tissues, whereas PPARγ is restricted mainly to adipose tissue, with some expression also in parts of the immune system, the retina, and other organs in trace amounts (25). The localization of PPARα and PPARγ gene transcripts and proteins and the nature of their target genes indicates that they play roles in fatty acid catabolism for PPARα, on the one hand, and adipogenesis and lipid storage for PPARγ, on the other hand (see Ref. 1 for a review). In contrast, however, the role of the ubiquitously expressed PPARβ remains elusive.

As the ubiquitous expression of PPARβ in the adult rat did not provide clear information as to its roles, developmental expression of this isotype was analyzed, with the goal of unraveling differential patterns of expression that would implicate PPARβ in specific events. Developmental patterns of expression of PPARα and PPARγ were also investigated to further understand their roles in the embryo. Previous reports of Northern blot analyses in *Xenopus* and mice showed that PPARβ is expressed early and throughout development, whereas PPARα and PPARγ appear only later in relatively high amounts (3, 7). However, these studies did provide only limited information, as the distribution within the embryo could not be resolved by the approach used. It is precisely to answer this question that analysis of the differential tissular expression of PPARs during embryonic development of the rat was undertaken by *in situ* hybridization. Rat embryos were studied using specific probes for rat PPARα, PPARβ, and PPARγ (25). Embryonic days (E) E8.5, E11.5, E13.5, E15.5, and E18.5 were chosen to cover the most important periods of development: E8.5 for the beginning of organization of the embryo in three layers; E11.5–E15.5 because of the intense differentiation processes that occur in most tissues, particularly in the central nervous system (CNS); and E18.5 because it represents a stage at which many tissues are already expressing specific functions that can be compared with those found in adulthood. Our results show that PPARα and PPARγ start to be expressed only late in

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Address all correspondence and requests for reprints to: Dr. Walter Wahli, Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. E-mail: walter.wahli@iba.unil.ch.
* This work was supported by the Etat de Vaud and the Swiss National Science Foundation.
† Present address: Laboratoire de Chimie Clinique, Centre Hospitalier Universitaire Vaudois, 1011-Lausanne, Suisse.

development (E13.5), mainly in the tissues where they will be found postnatally and in the adult. In addition, both PPARα and PPARγ present transient expression in the CNS around E13.5. In contrast to the specific distribution of PPARα and PPARγ, PPARβ is expressed ubiquitously and very early during embryogenesis, with a peak of expression in the developing nervous system on E13.5. During late development (E18.5), PPARβ decreases to levels that will be found later ubiquitously in adult tissues. This strikingly high expression of PPARβ during development points to potential new directions of investigation to analyze the to date elusive role of this PPAR isotype.

Materials and Methods

Complementary DNAs (cDNAs) and probes used for in situ hybridization

A cDNA comprising part of the D and E domains of the rat PPARα (nucleotides 1377–1766) (4) was obtained (25, 26). cDNAs comprising part of the A/B domain of the rat PPARβ (nucleotides 264–398) (8) and part of the A/B and C domains of the rat PPARγ (403 nucleotides long, 96% homologous to the mouse PPARγ) (23) were obtained (25). The three rat PPAR (α, β, and γ) cDNAs were subcloned in pBluescript KS^+ and SK^+ vectors (Stratagene, Heidelberg, Germany) and used to synthesize *in vitro* transcribed antisense and sense riboprobes (25, 27). Antisense and sense riboprobes were labeled with digoxigenin (Boehringer Mannheim, Mannheim, Germany).

Embryo preparation and in situ hybridization analysis

The age of Sprague-Dawley rat embryos (BRL, Basel, Switzerland) was determined from the appearance of a vaginal plug in pregnant females, and uteruses were dissected out. E8.5 embryos were let *in utero*, whereas E11.5, E13.5, E15.5, and E18.5 embryos were removed from the uterus and separated from the placenta. Uteruses (E8.5) and embryos (E11.5, E13.5, E15.5, and E18.5) were then quickly washed in diethylpyrocarbonate (Fluka, Buchs, Switzerland)-treated PBS and fixed 15 h in 4% paraformaldehyde-PBS at room temperature. Embryos were cryoprotected at 4 C in 12% sucrose-PBS and 18% sucrose-PBS for 6 and 12 h, respectively, and then embedded in tissue-freezing medium (Jung, Nussloch, Germany) and frozen in isopentane and dry ice. Embryos were kept at −80 C until used, and then cut and analyzed by *in situ* hybridization as previously described (25, 27). Briefly, cryosections 12 μm thick were prepared, and hybridization with digoxigenin-labeled antisense and sense riboprobes for rat PPARα, -β, and -γ was carried out at 60 C in 5 × SSC (standard saline citrate) and 50% formamide for 40 h. Washes (30 min in 2 × SSC at room temperature, 1 h in 2 × SSC at 65 C, 1 h in 0.1 × SSC at 65 C) and alkaline phosphatase staining were performed as previously described (25, 27). After staining, sections were dehydrated and mounted (Eukitt, O. Kindler Co., Freiburg, Germany). The specificity of hybridization was ascertained by the use of sense probes for the PPAR genes with the same length, GC content, and specificity as the corresponding antisense probes.

Histological analysis

In situ hybridization slides were observed and photographed on an Axiophot microscope (Carl Zeiss, Zurich, Switzerland).

Results

In a previous *in situ* hybridization study, we showed that the PPAR riboprobes we used were specific for each PPAR isotype (25). Moreover, we showed that the nonradioactive *in situ* hybridization protocol we used was sensitive enough to allow the detection of as few as 25 transcripts/cell (27).

PPARα

Transcripts of the PPARα gene were first detected at stage E13.5, where they were found at relatively high levels in the CNS (brain and spinal cord), tongue, and digestive tract (Fig. 1C). Lower levels were also detected in vertebrae, liver, and heart. On E15.5, PPARα expression decreased in the CNS, heart, digestive tract, and vertebrae (Fig. 2A), whereas it remained unchanged in the liver and was first detected in the epidermis, the cortex of the kidney, striated muscles, and the lung (Fig. 2C and Table 1). On E18.5, the PPARα gene was highly expressed in the liver (Fig. 3A and Table 1), the mucosa of the digestive tract (Fig. 3B and Table 1), BAT (Fig. 3C and Table 1), and epidermis (Fig. 3A and Table 1). PPARα transcripts were also well detected in the kidney cortex and pancreas (Fig. 3B and Table 1), but were barely detectable in muscle (Fig. 3, A and C) and CNS (not shown and Table 1). The staining of sections with a sense control probe for PPARα was negative at the different stages tested (Figs. 1D and 3D).

PPARβ

The PPARβ gene was expressed as early as E8.5 in the embryonic ectoderm, the visceral endoderm (Fig. 1E and Table 1), and the parietal endoderm (Fig. 1E and Table 1). On E11.5, PPARβ transcripts were present in the CNS (brain and spinal cord), the first branchial arch, and the bud of the digestive tract (Fig. 1F and Table 1). On E13.5, E15.5, and E18.5, PPARβ expression was ubiquitous, with relative levels of transcripts varying from one tissue to another (Figs. 1G; 2, D–F; and 3, E–I; and Table 1). An impressive peak of expression was observed between E13.5–E15.5 (Figs. 1F and 2D and Table 1), followed by a decrease on E18.5 (Fig. 3, E–I) to reach the levels of ubiquitous expression found in the adult rat (25). The peak of PPARβ transcripts was particularly high on E13.5 in the CNS (whole brain and spinal cord), the spinal ganglia, the tongue, the vertebrae, the liver, and the digestive tract. It was also clearly observed in the heart and epidermis, but at lower levels (Fig. 1G and Table 1). On E15.5, PPARβ was still very highly expressed in the CNS (whole brain and spinal cord), particularly in the forebrain (telencephalic cortex; Fig. 2, D and E, and Table 1). The liver, lung, digestive tract, and vertebrae also presented high levels of PPARβ messenger RNAs (mRNAs), whereas it was found at lower levels in epidermis, heart, pancreas, and squeletic muscle on E15.5 (Fig. 2, D and F, and Table 1). On E18.5, very high amounts of PPARβ transcripts were detected in the epidermis and BAT (Fig. 3, G and I, and Table 1), as well as in the follicles of vibrissae, primordia of teeth, and olfactory epithelium (Fig. 3I). It was also well expressed in the mucosa of the digestive tract, the cortex of the kidney, and the pancreas (Fig. 3F), as well as in squeletic muscle, heart, and lung (Table 1 and not shown). Interestingly at this stage, PPARβ expression was decreased in the liver (Fig. 3F and Table 1) and the CNS (Fig. 3E and Table 1). The staining of sections with a sense control probe for PPARβ was negative in the different stages tested (Figs. 1H, 2I, and 3H).

PPARγ

The expression of the PPARγ gene was first detected on E13.5, when relatively high transient levels of transcripts

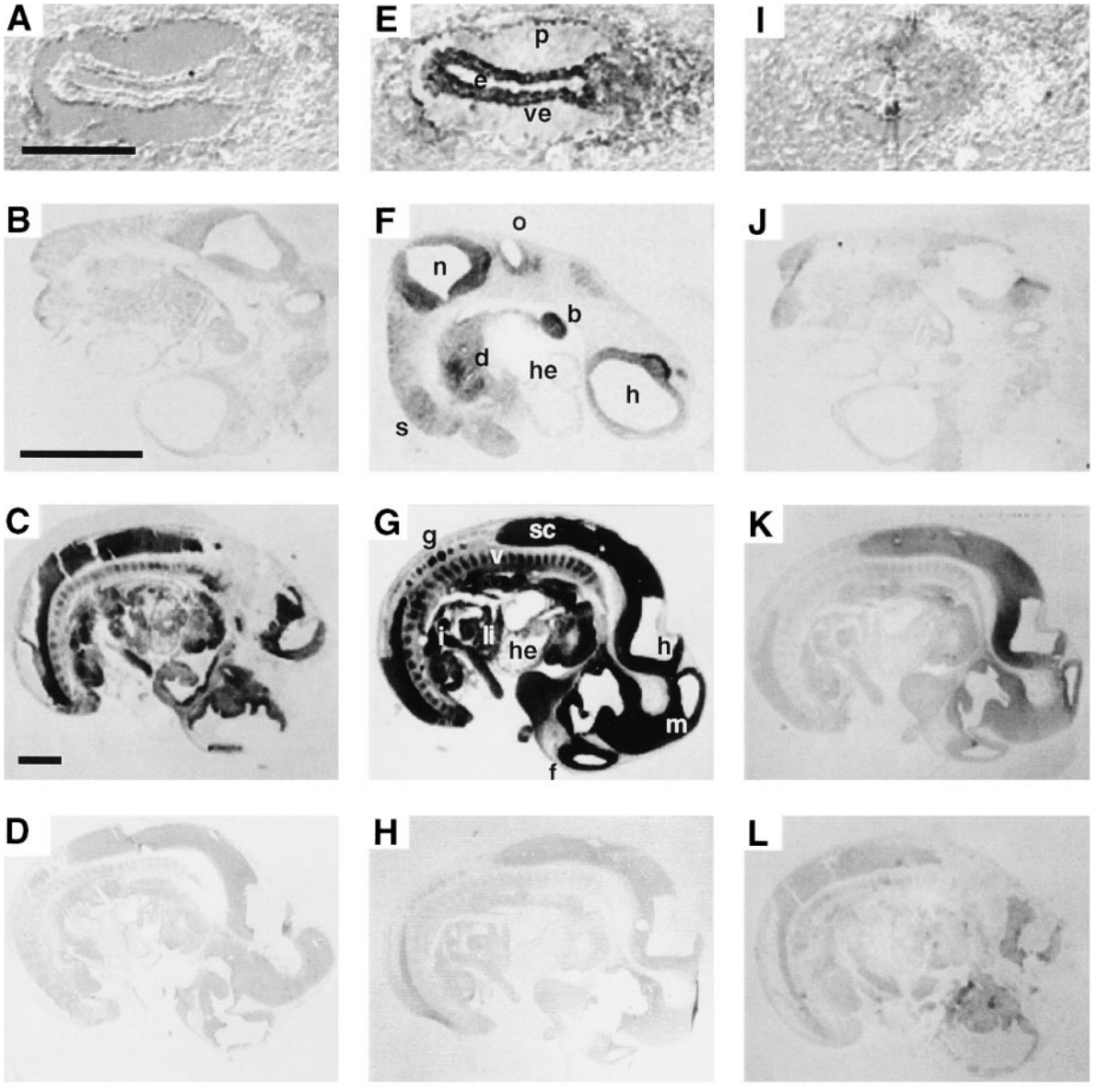

FIG. 1. Expression of PPARα, PPARβ, and PPARγ on E8.5, E11.5, and E13.5 in the developing rat embryo. *In situ* hybridization was performed with antisense probes for PPARα (A–C), PPARβ (E–G), and PPARγ (I–K), and sense probes for PPARα (D), PPARβ (H), and PPARγ (L). Whole embryos of E8.5 (A, E, and I), E11.5 (B, F, and J; sagittal), and E13.5 (C, D, G, H, K, and L; sagittal) were used. b, First branchial arch; d, digestive bud; e, embryonic ectoderm; f, forebrain; g, spinal ganglia; h, hindbrain; he, heart; i, intestine; li, liver; m, midbrain; n, neural tube; o, otic vesicle; p, parietal endoderm; s, somites; sc, spinal cord; v, vertebrae; ve, visceral endoderm. *Bars* = 200 μm (A, E, and I) and 1 mm (B–D, F–H, and J–L).

were observed in the CNS only, particularly in the hindbrain (Fig. 1K). On E15.5, the expression of PPARγ in the CNS was already reduced, and no other tissue was found expressing it (Fig. 2G and Table 1). On E18.5, PPARγ mRNAs were only detected in BAT, at very high levels (Fig. 3K and Table 1). The staining of sections with a sense control probe for PPARγ was negative at each stage tested (Figs. 1L, 2H, and 3L).

Discussion

This study establishes that the three PPAR isotypes are expressed differentially in numerous tissues originating from all three embryonic layers during rat development. PPARα and PPARγ appear late in development in the tissues where they will continue to be expressed in adulthood (25, 28), with the exception of a transient expression in the CNS (α and γ) and the epidermis (α). In contrast, PPARβ is already expressed ubiquitously at the earliest stage tested, with a marked peak on E13.5–E15.5, then declining to levels characteristic of adulthood (25).

Late expression of PPARα in embryogenesis

The late onset of PPARα expression (E13.5) in liver, cortex of kidney, intestinal mucosa, pancreas, and heart (this work and Ref. 29) correlates with the progressive differentiation of these organs (30), including the maturation of specific metabolic pathways in which PPARα has been found to be in-

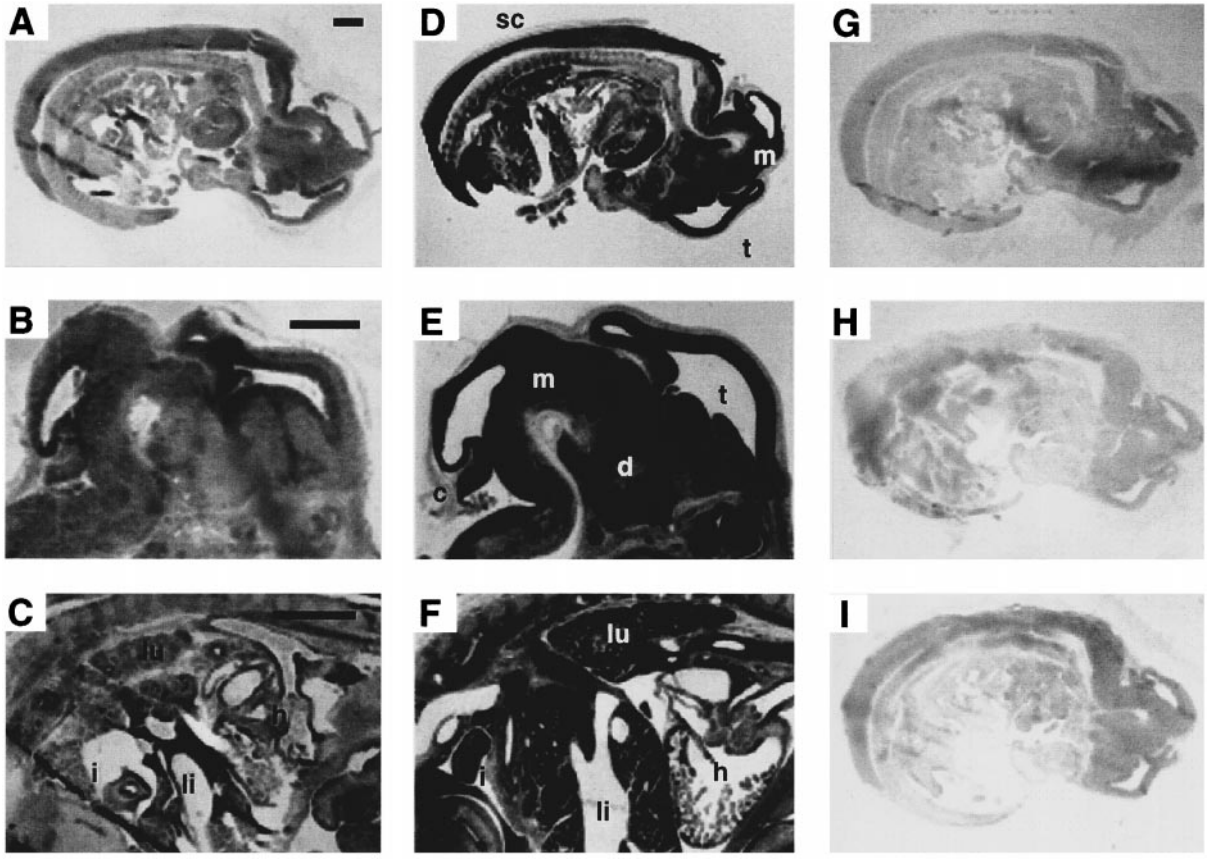

FIG. 2. Expression of PPARα, PPARβ, and PPARγ on E15.5 in the developing rat embryo. *In situ* hybridization was performed with antisense probes for PPARα (A–C), PPARβ (D–F), and PPARγ (G), and sense probes for PPARβ (I) and PPARγ (H). Whole embryos of E15.5 (A, D, and G–I; sagittal), and an enlargement of E15.5 brain (B and E; sagittal) and abdominal region (C and F; sagittal) are shown. c, Cerebellum primordium; d, diencephalon; h, heart; i, intestine; li, liver; lu, lung; m, mesencephalon; sc, spinal cord; t, telencephalon. *Bars* = 1 mm.

volved (see Ref. 1 for a review). Particularly, the expression of PPARα in the developing liver, kidney, and intestinal mucosa at the starting point of their differentiation makes it possible to regulate, early in histogenesis, the peroxisomal β-oxidation-encoding genes that are expressed in the primordials of these tissues before they differentiate (31). The expression of PPARα in rat fetal pancreas (E18.5) may indicate its involvement in insulin-secreting β-cells just before they change their metabolism from high fatty acid oxidation toward high glucose oxidation on E19.5 (32). PPARα knock-out mice develop normally and are fertile, without observable gross defects (33). Thus, either the role(s) of PPARα in metabolic pathways must be very subtle during embryogenesis, or compensatory events, *e.g.* by the other PPAR isotypes, are responsible for the apparently normal development of PPARα knock-out mice. PPARs could not be detected in the adult epidermal keratinocytes by the technique used herein (25). In contrast, we show in this study that PPARα and PPARβ are expressed in the rat embryonic epidermis. In the mouse embryo, PPARα and PPARβ mRNAs appear even earlier, together with PPARγ, which we did not observe in the rat (Michalik, L., and W. Wahli, personal communication). The earlier onset of PPARα and PPARβ in the mouse probably reflects the 2-day advance shift of mouse development compared with that in the rat (30), whereas the difference in PPARγ expression in embryonic epidermis as well as liver is illustrative either of differences between species or of the sensitivity of the methods used. However, it indicates that PPARs might be involved in the establishment of the skin lipid barrier, as suggested previously (34), based on a study using a mouse expressing a dominant negative retinoic acid receptor.

Transient expression of PPARα and PPARγ in the developing CNS

Interestingly, a transient peak of expression of PPARα is observed in the whole developing CNS, around the E13.5 stage. The same phenomenon is true for PPARγ, but is restricted to hindbrain. E13.5 corresponds to the onset of differentiation and apoptosis events in CNS, which would suggest a role for PPARα and PPARγ during these processes, but remains to be proven.

Coexpression of PPARα, -β, and -γ in BAT

White adipose tissue is specialized to store triglycerides and releases fatty acids upon demand, whereas BAT has the additional unique faculty to produce heat. This heat dissipation is made possible by the expression of a specific BAT protein, UCP, which uncouples the fatty acid oxidation from the synthesis of ATP (35, 36). Transgenic mice lacking BAT develop obesity (37), providing evidence for

TABLE 1. Differential expression of PPARα, PPARβ, and PPARγ during embryonic development of the rat

	E8.5	E11.5	E13.5	E15.5	E18.5
PPARα					
Embryonic ectoderm	−				
Visceral endoderm	−				
Parietal endoderm	−				
Forebrain		−	++	+	+/−
Midbrain		−	++	+	+/−
Hindbrain		−	++	+	+/−
Spinal cord		−	++	+	+/−
Spinal ganglia			++	ND	+/−
Epidermis		−	−	+	++
Kidney			ND	+	+
Liver		−	+	+	+++
Pancreas				+/−	+
Heart		−	+	+	−
Vertebrae			+	+/−	−
Squeletic muscle				+	+/−
Brown adipose tissue					++
Lung			ND	+	−
Intestine		−	++	++	++
PPARβ					
Embryonic ectoderm	++				
Visceral endoderm	++				
Parietal endoderm	+				
Forebrain		+	++++	++++	++
Midbrain		+	++++	+++	++
Hindbrain		+	++++	+++	++
Spinal cord		+	++++	+++	++
Spinal ganglia			++++	+++	++
Epidermis		−	+	+	+++
Kidney			ND	++	++
Liver		−	++	+++	+
Pancreas				++	++
Heart		−	+	++	++
Vertebrae			+++	+++	++
Squeletic muscle				++	++
Brown adipose tissue					++++
Lung			ND	+++	++
Intestine		+	++	+++	+++
PPARγ					
Embryonic ectoderm	−				
Visceral endoderm	−				
Parietal endoderm	−				
Forebrain		−	+	+/−	−
Midbrain		−	++	+/−	−
Hindbrain		−	+++	+/−	−
Spinal cord		−	++	+/−	−
Spinal ganglia			−	−	−
Epidermis		−	−	−	−
Kidney			ND	−	−
Liver		−	−	−	−
Pancreas				−	−
Heart		−	−	−	−
Vertebrae			−	−	−
Squeletic muscle				−	−
Brown adipose tissue					++++
Lung			ND	−	−
Intestine		−	−	−	−

− or + signs indicate differences in signal intensities observed by optical microscopy and reflect levels of PPAR expression. The number of + signs does not represent a strict linear measure of mRNA levels. −, Absent; +/−, barely detectable; +, weak expression; ++, moderate expression; +++, high expression; ++++, very high expression. ND, Not determined.

an important role of BAT in fatty acid catabolism. Furthermore, the specific expression of UCP in BAT is regulated by PPARγ (22), which also promotes BAT differentiation at the end of fetal life (28). Our results on PPARγ expression in embryonic rat BAT underscore and add to these data. Furthermore, we show that the two other isotypes, PPARα and PPARβ, are concomitantly expressed at high levels with PPARγ (this work and Ref. 29). This raises the question of the specific role(s) for each of the three receptors for BAT differentiation and functions. Can they all achieve the PPARγ function in this developing tissue as previously reported (28), or do PPARα and PPARβ have different specific roles? One would predict that as in adulthood, PPARγ is involved primarily in BAT differentiation and fatty acid uptake and storage, whereas PPARα would regulate BAT fatty acid catabolism. Finally, PPARβ would have a role in basal cellular metabolism, in line with its ubiquitous expression.

Potential roles of PPARβ

PPARβ is expressed ubiquitously, at higher levels during embryogenesis than in adulthood (this study and Ref. 25). It has been proposed as a repressor of other PPAR isotype activities (38), but its expression levels and pattern during development, compared with those of the α and γ isotypes, argue for a different function. Its ubiquitous expression suggests a specific role in each cell type, from the early embryo to adulthood, possibly under the control of specific ligands. One possibility could be a function in membrane lipid synthesis and turnover. Such a role is compatible with PPARβ expression in giant neurons of the adult brain (25), as these cells are characterized by huge dendritic trees and axons that need high amounts of membrane lipids and an efficient rate of membrane turnover both to maintain these structures and to remodel synaptic connections. Another possibility would be a function of PPARβ at the onset of differentiation processes. Indeed, we show that the peak of ubiquitous expression of PPARβ during embryogenesis correlates with that of the period of greatest cell differentiation activity (30), particularly in the CNS (39, 40). One hypothesis would view PPARβ as a sensor for a specific ligand that would induce cell differentiation, whereas the absence of ligand would direct the cell toward apoptosis by default, as previously suggested (36). This would be consistent with the PPARβ expression pattern in the adult, where it is found at high levels in tissues with a high rate of cell renewal and differentiation (25).

Conclusion

We have described the expression patterns of the three PPAR isotypes during embryonic development of the rat. PPARα and PPARγ appear late in development, in cells where they will be expressed later in adulthood, *e.g.* liver, intestine, kidney, and pancreas for α, and adipose tissue for γ. Furthermore, an interesting transient peak of expression of α and γ has been noticed in the CNS. The intriguing high coexpression of the three isotypes in the developing BAT and CNS provides opportunities to define their respective roles in these differentiating structures. Furthermore, the very strong ubiquitous PPARβ expression throughout development argues for a role of this isotype in basic cellular

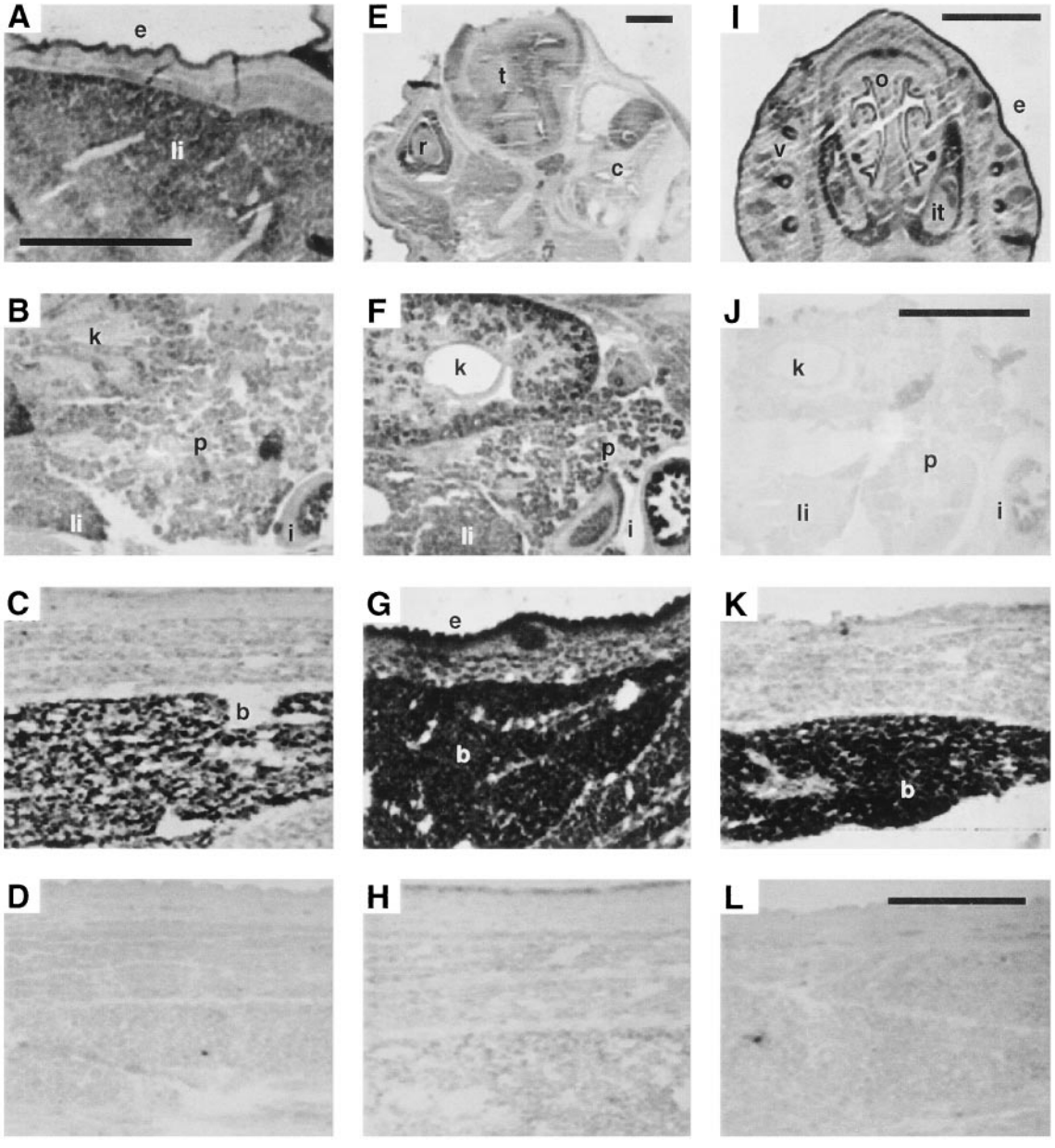

FIG. 3. Expression of PPARα, PPARβ, and PPARγ on E18.5 of the developing rat embryo. *In situ* hybridization was performed with antisense probes for PPARα (A–C), PPARβ (E–G and I), and PPARγ (J–K), and sense probes for PPARα (D), PPARβ (H), and PPARγ (L). Enlargements of E18.5 liver (A and B; sagittal), head (E; sagittal), abdominal region (F and J; sagittal), upper jaw (I; coronal), and BAT (C, D, G, H, K, and L; sagittal) are shown. b, Deposit of BAT; c, cochlea and semicircular canals; e, epidermis; i, intestine; it, primordium of upper incisor tooth; k, kidney; l, liver; o, olfactory epithelium; p, pancreas; r, retina; t, telencephalon; v, follicles of vibrissae. *Bars* = 1 mm (A, B, E, F, I, and J) and 200 μm (C, D, G, H, K, and L).

metabolic pathways, such as those involved in membrane synthesis and turnover, and possibly in cell cycle control.

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References

1. **Lemberger T, Desvergne B, Wahli W** 1996 Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol 12:335–363
2. **Issemann I, Green S** 1990 Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645–650
3. **Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W** 1992 Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68:879–887
4. **Göttlicher M, Widmark E, Li Q, Gustafsson JÅ** 1992 Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. Proc Natl Acad Sci USA 89:4653–4657
5. **Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA** 1992 Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Mol Endocrinol 6:1634–1641
6. **Sher T, Yi HF, McBride OW, Gonzalez FJ** 1993 cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. Biochemistry 32:5598–5604
7. **Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM** 1994 Differential expression and activation of a

family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci USA 91:7355–7359
8. **Xing G, Zhang L, Heynen T, Yoshikawa T, Smith M, Weiss S, Detera-Wadleigh S** 1995 Rat PPAR delta contains a CGG triplet repeat and is prominently expressed in the thalamic nuclei. Biochem Biophys Res Commun 217:1015–1025
9. **Amri EZ, Bonino F, Ailhaud G, Abumrad NA, Grimaldi PA** 1995 Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. J Biol Chem 270:2367–2371
10. **Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh, Greene G, Nimer SD** 1995 Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expression 4:281–299
11. **Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W** 1993 Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. Proc Natl Acad Sci USA 90:2160–2164
12. **Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA** 1995 An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J Biol Chem 270:12953–12956
13. **Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM** 1995 A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 83:813–819
14. **Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM** 1995 15-Deoxy-δ12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. Cell 83:803–812
15. **Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W** 1996 The PPARα-leukotriene B4 pathway to inflammation control. Nature 384:39–43
16. **Krey G, Braissant O, Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W** 1997 Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol Endocrinol 11:779–791
17. **Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM** 1997 Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc Natl Acad Sci USA 94:4318–4323
18. **Forman BM, Chen J, Evans RM** 1997 Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. Proc Natl Acad Sci USA 94:4312–4317
19. **Willson TM, Wahli W** 1997 Peroxisome proliferator-activated receptor agonists. Curr Opin Chem Biol 1:235–241
20. **Wahli W, Braissant O, Desvergne B** 1995 Peroxisome proliferator-activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more. Chem Biol 2:261–266
21. **Desvergne B, Wahli W** 1995 PPAR: a key nuclear factor in nutrient/gene interactions? In: Baeuerle P (ed) Inducible Transcription. Birkhaüser, Boston, vol 1:142–176
22. **Sears IB, MacGinnitie MA, Kovacs LG, Graves RA** 1996 Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. Mol Cell Biol 16:3410–3419
23. **Zhu Y, Alvarez K, Huang Q, Rao S, Reddy J** 1993 Cloning of a new member of the peroxisome proliferator activated receptor gene family from mouse liver. J Biol Chem 268:26817–26820
24. **Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM** 1994 mPPARγ2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 8:1224–1234
25. **Braissant O, Foufelle F, Scotto C, Dauça M, Wahli W** 1996 Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. Endocrinology 137:354–366
26. **Lemberger T, Staels B, Saladin R, Desvergne B, Auwerx J, Wahli W** 1994 Regulation of the peroxisome proliferator-activated receptor α gene by glucocorticoids. J Biol Chem 269:24527–24530
27. **Braissant O, Wahli W** 1998 A simplified *in situ* hybridization protocol using non-radioactively labelled probes to detect abundant and rare mRNAs on tissue sections. Biochemica 1:10–16
28. **Tai TC, Jennermann C, Brown KK, Oliver BB, MacGinnitie MA, Wilkison, WO, Brown HR, Lehmann JM, Kliewer SA, Morris DC, Graves RA** 1996 Activation of the nuclear receptor peroxisome proliferator-activated receptor γ promotes brown adipocyte differentiation. J Biol Chem 271:29909–29914
29. **Beck F, Plummer S, Senior PV, Byrne S, Green S, Brammar WJ** 1992 The ontogeny of peroxisome-proliferator-activated receptor gene expression in the mouse and rat. Proc R Soc Lond 247:83–87
30. **Kaufman M** 1992 The Atlas of Mouse development. Academic Press, Harcourt Brace Jovanovich, London, pp 1–512
31. **Keller JM, Cable S, el BF, Heusser S, Scotto C, Armbruster, Ciolek E, Colin S, Schilt J, Dauca M** 1993 Peroxisome through cell differentiation and neoplasia. Biol Cell 77:77–88
32. **Bliss CR, Sharp GW** 1994 A critical period in the development of the insulin secretory response to glucose in fetal rat pancreas. Life Sci 55:423–427
33. **Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ** 1995 Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 15:3012–3022
34. **Imakado S, Bickenbach JR, Bundman DS, Rothnagel JA, Attar PS, Wang, XJ, Walczak VR, Wisniewski S, Pote J, Gordon JS** 1995 Targeting expression of a dominant-negative retinoic acid receptor mutant in the epidermis of transgenic mice results in loss of barrier function. Genes Dev 9:317–329
35. **Nicholls DG, Locke RM** 1984 Thermogenic mechanisms in brown fat. Physiol Rev 64:1–64
36. **Klaus S, Casteilla L, Bouillaud F, Ricquier D** 1991 The uncoupling protein UCP: a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. Int J Biochem 23:791–801
37. **Lowell BB, Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer, BB, Kozak LP, Flier JS** 1993 Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 366:740–742
38. **Jow L, Mukherjee R** 1995 The human peroxisome proliferator-activated receptor (PPAR) subtype NUC1 represses the activation of hPPAR α and thyroid hormone receptors. J Biol Chem 270:3836–3840
39. **Jacobson M** 1991 Developmental Neurobiology. Plenum Press, New York, pp 1–465
40. **Altman J, Bayer S** 1995 Atlas of Prenatal Rat Brain Development. CRC Press, Boca Raton, Ann Arbor, London, pp 1–589