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Review

Implications of Peroxisome Proliferator- Activated Receptors (PPARS) in development, cell life status and disease

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Introduction

Dietary fatty acids are important nutrients for the growth, development and physiology of vertebrates. They represent an energy source for the cells in which they are transported, interconverted, stored, mobilized and used. They also participate in the synthesis of essential membrane components. In addition, fatty acids derived from nutrition and/or endogenous metabolism can act as hormones in regulating gene expression. Several specific fatty acid-regulated transcription factors have been identified and studied extensively. The discovery of a novel receptor that was activated by fatty acid-like chemicals called peroxisome proliferators (PPs) (Issemann and Green, 1990) has added yet another signaling molecule to the arsenal utilized by fatty acids and their metabolites to control gene expression. The object of this paper is to describe in detail selected aspects of our understanding about the involvement of peroxisome proliferator-activated

Abbreviations used in this paper: AOX, acyl coenzyme a oxidase; AP-1, activating protein 1; ARP-1, apolipoprotein regulatory protein 1; CNS, central nervous system; COUP-TFI, chicken ovalbumin upstream promoter transcription factor I; DR, Direct repeat; HNF-4, hepatocyte nuclear factor-4; LTB4, leukotriene B4; NF-κB, nuclear factor-kappa B; Ox-LDL, oxidized very low-density lipoprotein; PGI2, prostacyclin I2; PGJ2, prostaglandin J2; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; mPPAR, mouse peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-response element; RAR, retinoic acid receptor; RXR, 9-*cis* retinoic acid receptor; STAT, signal transducer and activator of transcription; THR, thyroid hormone receptor; TNFα, tumor necrosis factor alpha; VLDL, very low-density lipoprotein.

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receptors (PPARs) in development and cell life status, and to define how these transcription factors exert pivotal roles in health and disease.

From peroxisomes to PPARs

Peroxisomes are ubiquitous cellular organelles bound by one unit membrane (Fig. 1). Their size, abundance and enzyme composition vary considerably depending on the species and cell type in which they are encountered (see Roels, 1991; Masters and Crane, 1995). These cytoplasmic corpuscles are characterized biochemically by the presence of H_2O_2-generating oxidases and a H_2O_2-destroying catalase (De Duve and Baudhuin, 1966). The diverse metabolic pathways in which these structures are involved have been mainly investigated in mammalian hepatocytes (see Van den Bosch *et al.,* 1992). Enzymes present in liver peroxisomes are associated with lipid metabolism, namely with β-oxidation of very long-chain fatty acids and of biologically important lipid derivatives such as prostaglandins and leukotrienes. They play a role in plasmalogen and cholesterol synthesis. Peroxisomes are also involved in the catabolism of polyamines, pipecolic acid, purines and glyoxylate. The essential role exerted by peroxisomes in cellular metabolism is emphasized by the identification of a group of human genetic diseases that have a number of metabolic disturbances considered to be due to peroxisomal defects. The failure to form or maintain the peroxisome or a defect in the function of a single enzyme that is normally in this organelle results in serious diseases (Singh, 1997).

Since the earliest ultrastructural observations by Paget (1963) of livers of rats fed clofibrate, it has been well documented that this hypolipidemic agent and other compounds related to fibrate drugs induce peroxisome proliferation in rodents (Hawkins *et al.,* 1987; Fig. 1). In addition to fibrates, a range of xenobiotic compounds, such as phthalate and adipate ester plasticizers, herbicides, leukotriene antagonists, acetylsalicylic acid, thio-substituted and perfluorinated fatty acids also induce peroxisome proliferation in rodents. Despite the diversity of their structures, most PPs contain a hydrophobic domain associated with a carboxylic acid group. Pathophysiological conditions characterized by modifications in body fatty acid amounts such as after increased dietary fat intake, or in times of starvation (Thomas *et al.,* 1989; Orellana *et al.,* 1992), or during uncontrolled diabetes mellitus (Thomas *et al.,* 1989) induce cellular effects which are very similar to those caused by PPs. Peroxisome proliferation is a pleiotropic cellular response which involves drastic changes in both morphology, number and enzyme activity of peroxisomes (Lock *et al.,* 1989). Modifications of non-peroxisomal enzymatic activities are also noted in the livers of those PP-treated animals (Masters and Crane, 1995). In addition, peroxisome proliferation is accompanied by hepatocyte proliferation and liver growth. However, the response to PPs varies depending on the species examined as rats and mice are highly sensitive while guinea pigs, monkeys and humans are relatively refractory.

As PPs are lipophilic signaling molecules exerting their regulatory functions at the genome level, it was postulated that their action could be mediated by intracellular receptors. A screen for novel nuclear receptor cDNAs in the liver of fibrate-treated mice identified a clone encoding a molecule which was able to be activated by PPs in a transient transfection assay (Issemann and Green, 1990). This receptor was called the mouse peroxisome proliferator-activated receptor (mPPAR).

PPAR characteristics

Since this discovery, four isotypes of PPAR have been described, known as α, β also called δ or NUC1, and γ (Dreyer *et al.,* 1992; Schmidt *et al.,* 1992; Kliewer *et al.,* 1994). The latter exists as two isoforms, γ_1 and γ_2, resulting from alternative promoter usage and differential splicing (Zhu *et al.*, 1995). The PPARs form a distinct subfamily within the superfamily of nuclear receptors. They are organized into several structural and functional domains (Fig. 2). The NH2-terminal A/B domain which is the least conserved

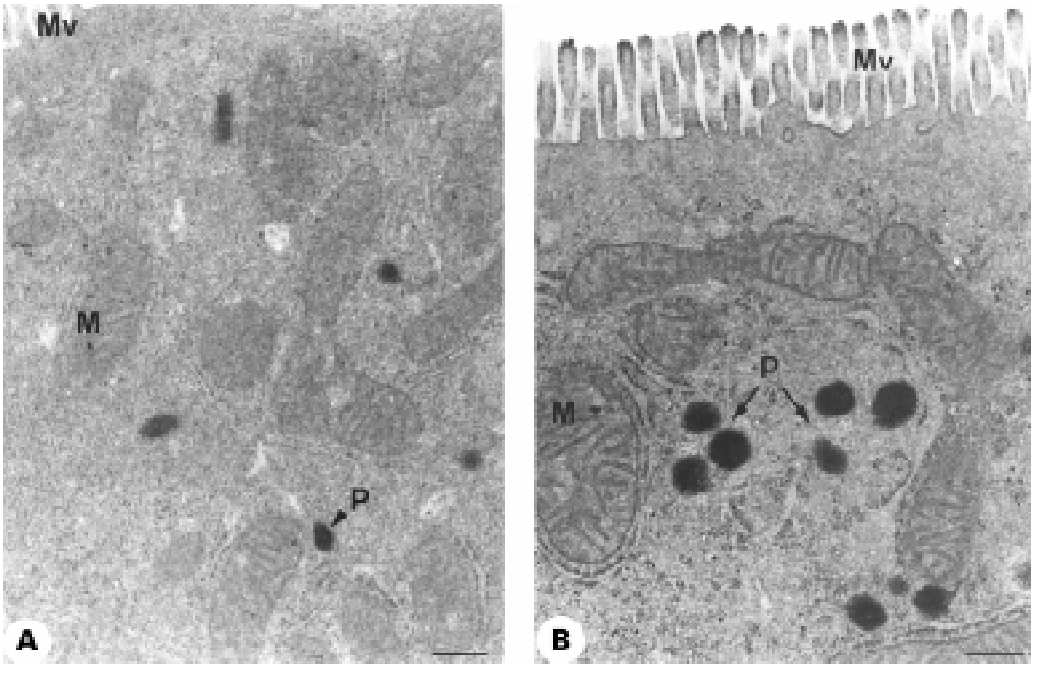

Fig. 1. Peroxisome proliferation in mouse duodenal explants cultured in the presence of clofibrate. *Duodenal explants from 15-day-old fetal mouse were cultured for 48 h in Trowel T8-medium supplemented with an organic extract of amniotic fluid, without* **(A)** *or with addition* **(B)** *of 500 μM clofibrate. Clofibrate induces an increase in the number and size of peroxisomes in epithelial cells. Bars represent 0.6 μm for* **(A)** *and 0.35 μm for* **(B)**. *M, mitochondria; Mv, microvilli; P, peroxisomes.*

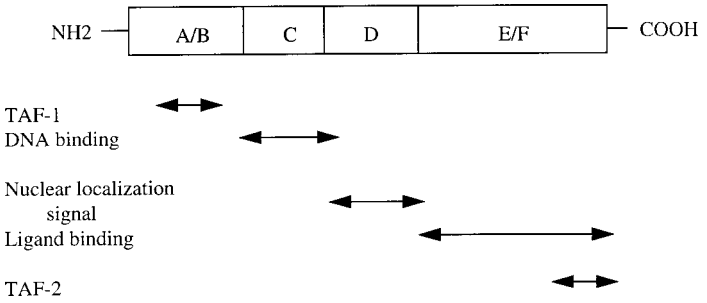

Fig. 2. Molecular organization of the functional domains within the PPAR subtypes. *This organization is similar to that of other nuclear hormone receptors. Biochemical and genetic data have provided insights about the boundaries and functions of the major PPAR domains. TAF-2 and TAF-1 are regions of the receptors involved in a ligand-dependent and -independent transcriptional activation function, respectively.*

region, contains a constitutive ligand-independent transactivation function. The well conserved DNA-binding domain C targets the receptor to specific DNA sequences, known as PPAR responsive elements (PPREs) ; this domain exhibits two zinc finger complexes each containing a conserved amino acid stretch. The P box in the first finger is involved in specific recognition of the PPRE, and the D box in the second finger participates in heterodimerization. The D box of PPARs contains only three amino acids instead of five amino acids in most nuclear receptors. The hinge or D domain allows interaction of the protein with co-activators and repressors. The large E/F domain is multifunctional as in addition to ligand binding, it is required for nuclear localization, heterodimerization and ligand-dependent transactivation. The PPAR carboxy terminus confers the subtype-specific responsiveness to the various activators.

The diversity of the PPAR activators has led to the search for the true ligands. Certain poly- and monounsaturated fatty acids bind to PPAR but the former are stronger activators (Göttlicher *et al.,* 1992; Keller *et al.,* 1993; Bocos *et al.,* 1995; Kliewer *et al.,* 1997). Linolenic acid activates mouse PPARα and PPARβ/δ but not PPARγ (Kliewer *et al.,* 1994). Some fatty acid metabolites bind to PPAR with higher affinity than their precursors. Recently, the (9Z,11E)-conjugated linoleic acid has been found to be a potent naturally occurring ligand and activator of PPARα (Moya-Camarena *et al.,* 1999) so are the 9- and 13-hydroxyoctadecadienoic acids, both metabolic derivatives of linoleic acid, for PPARγ (Nagy *et al.*, 1998). Some arachidonic acid metabolites such as the leukotriene B4 (LTB4) and the prostaglandin J2 (PGJ2) derivative, 15 deoxy-Δ12,14 PGJ2, represent natural ligands for PPARα (Devchand *et al.,* 1996) and PPARγ (Forman *et al.,* 1995; Kliewer *et al.,* 1995), respectively. Synthetic pharmacological products are also potent PPAR activators. The fibrate drugs used for their hypolipidaemic properties bind mainly to PPARα (Krey *et al.,* 1997). The antidiabetic thiazolidinedione agents which cause insulin sensitivity and hyperglycemic effects in animal models of non-insulin-dependent diabetes mellitus, are high-affinity ligands for PPARγ (Lehmann *et al.,* 1995).

The PPARs exhibit different tissue distributions. PPARα is mainly expressed in liver, kidney, heart, white and brown adipose tissues which exhibit high fatty acid metabolism and high peroxisome-dependent activities (Kliewer *et al.,* 1994; Mukherjee *et al*., 1994; Braissant *et al.,* 1996; Auboeuf *et al*., 1997). PPARβ/δ is expressed ubiquitously in all rodent and human tissues tested and often at higher levels than PPARα and γ, except in liver where the level of this receptor is low (Braissant *et al.,* 1996; Auboeuf *et al.,* 1997). PPARγ predominates in adipose tissue , spleen and large intestine (Kliewer *et al.,* 1994; Tontonoz *et al.,* 1994a; Auboeuf *et al.,* 1997). While PPARγ1 exhibits a tissue distribution pattern reminiscent of PPARα, PPARγ2 is mainly expressed in adipose tissue.

As for most nuclear hormone receptors, heterodimerization of PPAR is required before DNA binding. The preferential partner is the 9-*cis* retinoic acid receptor (RXR) (Issemann *et al.,* 1993; Keller *et al.,* 1993). A number of nuclear receptors, including retinoic acid receptors (RARs), 9-*cis* retinoic acid receptors (RXRs), hepatocyte nuclear factor-4 (HNF-4), chicken ovalbumin upstream promoter transcription factor I (COUP-TFI), apolipoprotein regulatory protein 1 (ARP-1) bind to response elements comprised of two core motifs, 5'-RG(G/T)TCA, or a closely related sequence separated by 1nt (Direct Repeat elements with one spacing base or DR1). PPARs mostly interact with the peroxisome proliferator response element (PPRE) corresponding to a DR1 with the 5'-AGGTCA-3' consensus motif (Tables 1 and 2). The potential role of the precise sequence of the core motif as well as the spacer nucleotide are important in determining specificity and promiscuity of receptor-response element interactions. It has been shown that nucleotides at base positions 1, 2 and 4 of the core motif as well as the spacer nucleotide determine the binding preference of PPAR/RXR and RAR/RXR heterodimers and HNF-4 and ARP-1 homodimers (Nakshatri and Bhat-Nakshatri, 1998). These authors have demonstrated in transfection experiments that transcriptional activa-

TABLE 1

COMPARISON OF IDENTIFIED *PPRE* SEQUENCES

Gene	Species	Element	Sequence	Protein function	Ref
Malic enzyme	Rat	Mep	TTCT GGGTCA A AGTTGA	Malate decarboxylation providing NADPH for fatty acid synthesis	(1)
Liver fatty acid binding protein	Rat	LFABP	ATAT AGGCCA T AGGCCA	Fatty acid binding	(2)
Adipocyte lipid binding protein (aP2)	Mouse	ARE 7	TCAT GGATCA G AGTTCA	Lipid binding	(3)
Fatty acid transport protein	Mouse	FATP	AAGT GGGGCA A AGGGCA	Fatty acid transport	(4)
Phospholipid transfer protein	Human	PPAR / PLTP	AAGT GACTTG (N4)AGATCA	Transfer of phospholipids to HDL	(5)
Muscle-type carnitine palmitoyl transferase	Human	MCPT I	ATGT AGGGAA A AGGTCA	Fatty acid transport	(6)
Acyl-CoA binding Protein	Rat	ACBP	AGTGCA A AGGTGA	Acyl-CoA binding	(7)
Acyl-CoA Synthase	Rat	ACS (CI)	TTTC AGGGCA T CAGTCA	Fatty acid activation	(8)
Acyl-CoA Oxidase	Rat	ACO (A)	GACC AGGACA A AGGTCA	Peroxisomal β-oxidation	(9)
		ACO (B)	AGCA AGGTAG A AGGTCA		(10)
	Human	hACOX	TAGA AGGTCA G CTGTCA		(11) (12)
Peroxisomal bifunctional enzyme	Rat	BIF	ATGT AGGTAA T AGTTCA	Peroxisomal β-oxidation	(13)
Consensus			AACT AGGGCA A AGGTCA Tg g T		

References in parentheses are as follows : (1) Castelein *et al.,* 1994; (2) Issemann *et al.* 1992; (3) Tontonoz *et al.,* 1994a; (4) Frohnert *et al.,* 1999; (5) Tu and Albers, 1999; (6) Mascaro *et al.,* 1998; (7) Elholm *et al.,* 1996; (8) Schoonjans *et al.,* 1995; (9) Osumi *et al.,* 1991; (10) Tugwood *et al.,* 1992; (11) Varanasi *et al.,* 1998; (12) Woodyatt *et al.,* 1999; (13) Zhang *et al.,* 1992. In the consensus sequence derived from listed PPREs, the uppercase letters denote most conserved base(s) and the lowercase letters indicate a less conserved base alternative.

tion by PPAR/RXR and HNF-4 and repression by ARP-1 correlate with the relative *in vitro* affinity provided the element was located with the proper promoter context. Furthermore, promoter context also determines whether an element that binds to PPAR/RXR and HNF-4 with equal affinity functions as a PPRE or an HNF-4 response element (Hertz *et al.,* 1998; Nakshatri and Bhat-Nakshatri, 1998). Thus, apart from the element-specific differences in affinity for the receptors, additional promoter-specific transcription factors that interact with PPAR/RXR and HNF-4 determine the specificity of transcriptional response through DR1-type elements. The fact that the PPAR/RXR heterodimer also binds *in vitro* to the palindromic estrogen responsive element AGGTCAnnnTGACCT (Keller *et al.,* 1995) is of particular biological interest as it suggests that fatty acids and PPs could interfere with the estrogen signaling pathway. In addition, RXR (Kliewer *et al.,* 1992) as well as PPAR (Bogazzi *et al.,* 1994) interact with thyroid hormone receptor (THR). Together these data indicate that crosstalk exists between PPAR, RXR and THR. As retinoids and thyroid hormones are important in development, PPAR could participate in the execution of the developmental program.

PPARs and development

Spatio-temporal expression of PPARs during mouse development

To study PPAR expression during mouse embryonic development, we performed immunohistochemistry with specific antibodies described in Huin *et al.* (2000). Mating of Swiss ICR mice was accomplished by leaving 1 male with 4 females overnight. Time of conception was fixed at 9: 00 a.m. the next morning, at which time males were removed from the cages. Hence, the time of conception was estimated with a possible error of 12h. Pregnant females at different days of gestation were anesthetized with ether. Embryos or fetuses were removed and fixed for 24h at 4°C by immersion in acetic acid-free Bouin-Holland fixative supplemented at the time of use with 10% neutral formol and 10% of an aqueous saturated solution of HgCl2.. Specimens were then washed in water all day long, dehydrated in a graded series of ethanol and finally washed in toluene. After embedding in paraffin, 5 μm thick sections were cut and mounted on slides. For immunostaining, the deparaffinized and rehydrated sections were immersed in 100 mM glycine in phosphate buffer saline (PBS) for 45 min at 4°C, then washed in PBS. The sections were preincubated with a blocking solution containing 0.1% fish gelatin, 0.8% bovine serum albumin (BSA) and Tween-80 (2 μl/100 ml PBS) for 30 min at room temperature (RT_0). They were then exposed to the primary antibody (diluted 1: 250 in PBS/BSA 2%) for 60 min at RT_0. After two washes in PBS, sections were exposed to the secondary antibody (1: 50 in PBS/BSA 2%), fluorescein-conjugated goat anti-rabbit IgG (Boehringer Mannheim Biochemica, Mannheim, Germany), for 60 min at RT_0. Negative controls were performed by replacing the primary antibody with PBS or with preimmune serum. Sections were stained with $2^0/_{00}$ Evans blue then mounted in Vectashield medium and photographed with a Reichert/Jung Polyvar microscope (Vienna, Austria).

TABLE 2

COMPARISON OF IDENTIFIED *PPRE* SEQUENCES

Gene	Species	Element	Sequence	Protein function	Ref
Medium-chain acyl-CoA dehydrogenase	Human	MCAD	TGACCT (N8)GGGTAA AGGTGA(N4)TGACCA	Mitochondrial fatty acid β–oxidation	(14)
Cytochrome P450A1	Rat	CYP4A1	AACT AGGGTA A AGTTCA	ϖ–Oxidation	(15)
Cytochrome P450A6	Rabbit	CYP4A6(Z)	AACT AGGGCA A AGTTGA	ϖ–Oxidation	(16)
3-Hydroxy-3-methylglutaryl-CoA synthase	Rat	HMG	AACT GGGCCA A AGGTCT	Liver ketogenesis and	(17)
	Pig		ATCT ATGTGA A AGGTCT	sterol synthesis	(18)
Phosphoenol pyruvate carboxy-kinase	Rat	PCK1 PCK2	CCCA CGGCCA A AGGTCA AACT GGGATA A AGGTCT	Glycerogenesis and glucogenesis	(19)
Lipoprotein lipase	Rat	LPL	AAGA GGGGGA A AGGGCA	Hydrolysis of triglyceride rich particles	(20)
Apolipoprotein A-I	Human	APOAI	T AGGGCA G GGGTCA AG	Protein component of HDL	(21)
Apolipoprotein A-II	Human	APOAII	TACC AGGGTA A AGGTTG	Protein component of HDL	(22)
Apolipoprotein C-III	Human	APOCIIIB	GCGC TGGGCA A AGGTCA	Triglyceride clearance	(23)
Uncoupling protein 1	Mouse	URE1	AGTG TGGTCA A GGGTGA	Thermogenesis	(24)
Cu/Zn superoxide dismutase	Rat	SOD/PPRE	CTGG GAGGCA G AGGTCA	Dismutation of superoxide radicals	(25)
Consensus			AACT AGGGCA A AGGTCA Tg g T		

References in parentheses are as follows : (14) Gulick *et al.,* 1994; (15) Aldridge *et al.,* 1995; (16) Muerhoff *et al.,* 1992; (17) Rodriguez *et al.,* 1994; (18) Ortiz *et al.,* 1999; (19) Tontonoz *et al.,* 1995; (20) Schoonjans *et al.,* 1996; (21) Vu-Dac *et al.,* 1994; (22) Vu-Dac *et al.,* 1995; (23) Hertz *et al.,* 1995; (24) Sears *et al.,* 1996; (25) Yoo *et al.,* 1999. In the consensus sequence derived from listed PPREs, the uppercase letters denote most conserved base(s) and the lowercase letters indicate a less conserved base alternative.

The PPAR proteins display widely different spatio-temporal patterns of expression during mouse development. The presence of the different PPAR subtypes was detected in the mouse blastocyst as soon as day 5 post conception (Figs. 3-5A). At midgestation, the PPARα protein was found in the tongue, liver, digestive tract, heart and vertebrae (Fig. 3 B-D). At this stage, the PPARβ/δ protein was ubiquitously expressed in most organs of the mouse embryo (Fig. 4 B-D). The presence of this PPAR subtype was clearly observed in the central nervous system (CNS), skeletal muscle, epidermis, lung and liver. It was also found at lower levels in the heart and the mucosa of the digestive tract (Fig. 4C), but was not found in the vertebrae (Fig. 4B). At the same time, PPARγ was very highly expressed in the hindbrain, the spinal cord, the vertebrae, the heart and the brown adipose tissue (Fig. 5 B-D).No immunoreactivity was found in control sections when the primary antibody was omitted or replaced by preimmune serum (not shown).

Comparison with other vertebrate developments

Few investigations have been performed on the spatio-temporal expression of the PPAR genes during vertebrate development. Most of these studies have been carried out by Northern blot analysis or *in situ* hybridization.

PPARα and PPARβ are expressed throughout oogenesis and embryogenesis of *Xenopus laevis* while PPARγ could not be detected until post-embryonic stages (Dreyer *et al.,* 1992). The steady-state amount per oocyte of PPARβ maternal mRNA is reached before accumulation of rRNA. In contrast, the time course of accumulation of PPARα is comparable with that of rRNA. Zygotic transcripts start to accumulate during neurula stages for PPARβ and later at tailbud stages for PPARα. Throughout the amphibian embryonic development, PPARβ mRNA is much more abundant than PPARα mRNA.

Our results showing that the different PPAR proteins are present in the mouse blastocyst as soon as day 5 post conception are somewhat at variance with those obtained for the corresponding transcripts during mouse embryonic development. These transcripts were not detected until day 9.5 for PPARβ/δ and day 13.5 for PPARα and PPARγ through Northern analysis of whole mouse embryo RNA (Kliewer *et al.,* 1994) or by *in situ* hybridization (Beck *et al.,* 1992). The high sensitivity of the immunohistochemical technique could explain this discrepancy. Recently, Braissant and Wahli (1998) have analyzed the expression of PPARs in the rat embryo by *in situ* hybridization. They found that PPARβ/δ transcripts appeared very early during embryogenesis with a peak of expression in the developing CNS at day 13.5. The distribution of PPARβ/δ mRNA was ubiquitous with relative levels varying from one tissue to another. On the other hand, according to these authors the presence of PPARα and PPARβ/δ mRNA was detected only in late development mainly in the tissues where they will be found postnatally and in the adult (Braissant *et al.,* 1996). In addition, both PPARα and PPARγ present transient expression in the CNS around day 13.5. Thus, the three PPAR isotypes are expressed differentially during murine development suggesting that these receptors play distinctive roles. PPARα expression is mainly observed in differentiating cells which will exhibit a high level of peroxisomal β-oxidation activity in adulthood. However, as null mutant mice lacking PPARα protein are viable, fertile and morphologically normal (Lee *et al.,* 1995), PPARα does not seem essential for rodent development and fertility. Until now, assays to produce mice nullizygous for PPARβ/δ or PPARγ have failed suggesting that the disruption of these genes is lethal. Furthermore, indirect data suggest that these receptors should be involved in embryo implantation. Using cyclo-oxygenase-2-deficient mice, Lim *et al.* (1999) have shown that the biosynthesis of the prostacyclin PGI2 is essential for mouse blastocyst implantation and decidualization and that the PGI2 effects are mediated by the activation of PPARδ. In addition, PPARγ $^{-/-}$ conceptuses exhibit a placental agenesis (Barak *et al.,* 1999) which has been reported to be similar, albeit less severe, than that observed during the postimplantation period of RXRα/RXRβ double null mouse embryos causing their death (Wendling *et al.,* 1999). According to these authors, the formation of the chorioallantoic placenta requires the participation of RXRs through heterodimerization with probably PPARγ. These data argue for an early expression of PPARγ and are in line with our immunocytochemical observations.

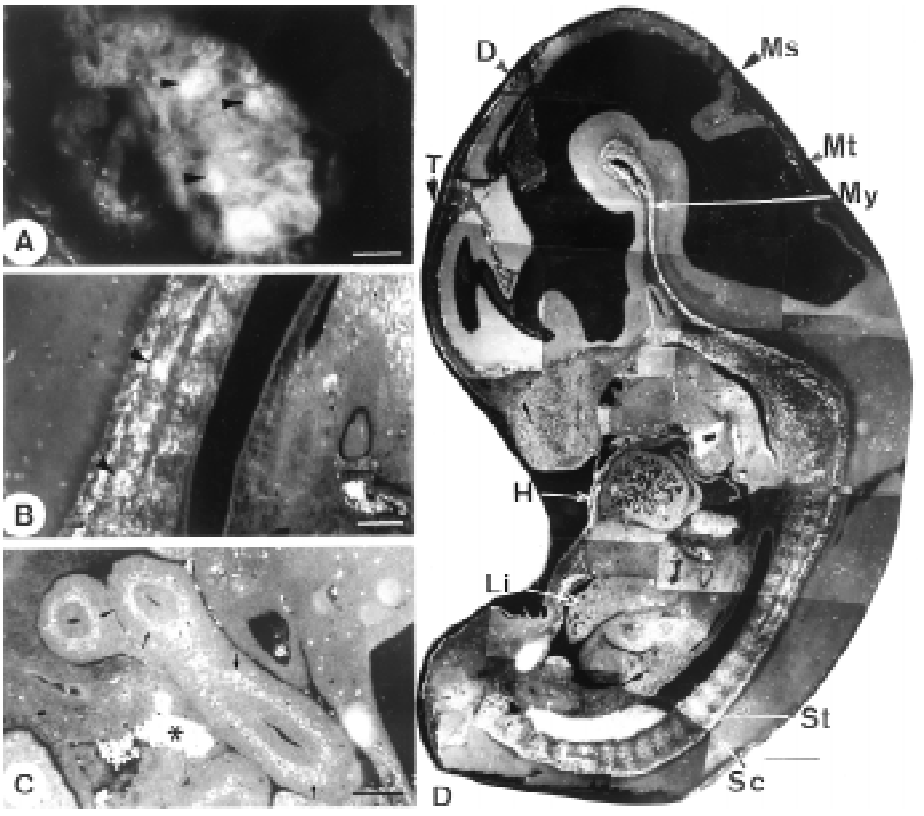

Fig. 3. Localization of PPARα in mouse embryos by indirect immunofluorescence. *The spatio-temporal distribution of PPARα was investigated during mouse embryonic development, using a specific polyclonal antibody characterized by Huin et al., 2000.* **(A)** *5 days of gestation. Some nuclei of the mouse blastocyst are stained (arrowheads). Bar, 16 μm.* **(B)** *Details of the sclerotomes of an 11-day-old mouse embryo. The labeling is intense in nuclei of cells participating to the development of the vertebrae (arrowheads). Bar, 150 μm.* **(C)** *Sections of the intestinal tract from a 13-day-old embryo showing reactive nuclei in the epithelium (arrows). The strong reaction in the structures (*) surrounding the intestinal loops is due to an artifact as it is also observed in control sections. Bar, 160 μm.* **(D)** *Parasagittal section of a gestation day-11 mouse embryo. Bar, 400 μm. D, diencephalon; H, heart; Li, liver; Ms, mesencephalon; Mt, metencephalon; My, myelencephalon; Sc, spinal cord; St, sclerotome; T, telencephalon.*

PPARγ is expressed in human trophoblast cells and its activation stimulates chorionic gonadotropin synthesis (Malassiné *et al.,* 2000). We have reported (Huin *et al.*, 2000) that the different PPAR proteins are expressed very early, as soon as 7 weeks of gestation, in the human fetal digestive tract. The PPAR subtypes are detected in both cell types of endodermal and mesodermal origin. PPARα, PPARβ and PPARγ exhibit different patterns of expression during morphogenesis of the digestive tract. Whatever the stage and the gut region (except the stomach) examined, PPARγ is expressed at a high level suggesting some fundamental role for this receptor in development and/or physiology of the human digestive tract.

Co-expression of PPARs and RXRs

RXRs (Dolle *et al.,* 1994) and PPARs share large common spectra of expression during murine embryogenesis. The early embryo development and placentogenesis need the expression of both PPARβ/δ, PPARγ as well as that of RXRα and RXRβ. Both PPARβ/δ and RXRβ are found in almost all tissues and this embryonic pattern perdures in the adult organism. On the other hand, the α- and γ-subtypes of these receptors are much more restricted. The expressions of PPARα and RXRα are abundant in the liver, kidney and a variety of visceral tissues. Both PPARs are expressed with different levels in the spinal cord as do RXRs. Thus, the spatio-temporal co-localization of PPARs and RXRs suggests the participation of their heterodimers in the program of development.

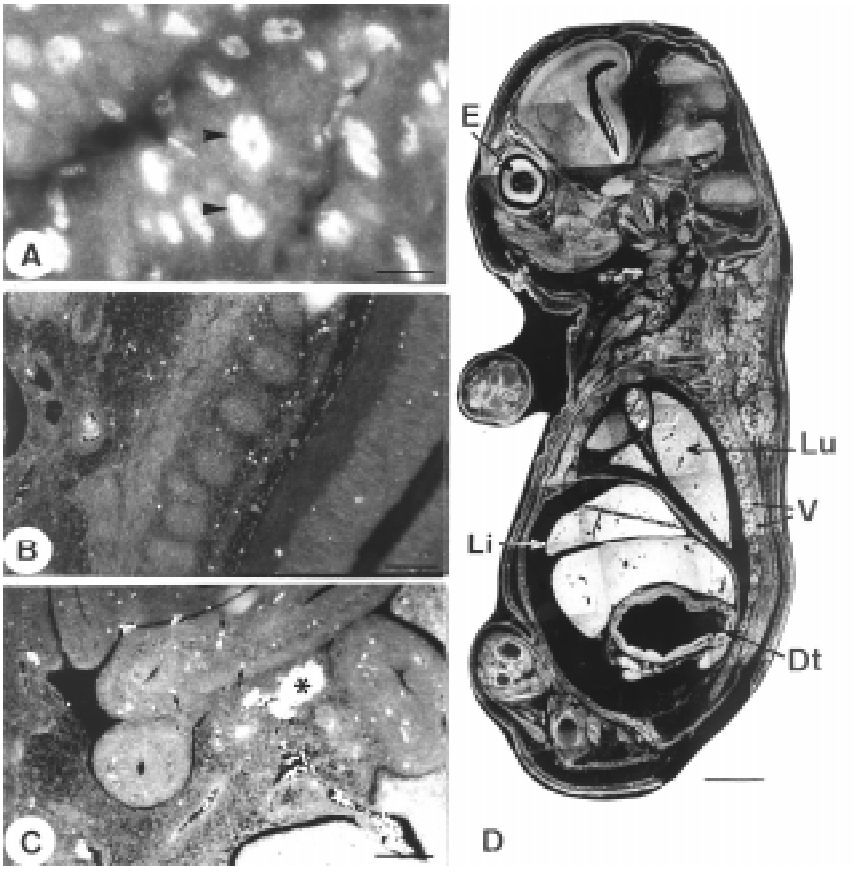

Fig. 4. Developmental expression of PPARβ/δ in the mouse embryo. *The polyclonal antibody raised against PPARβ/δ which was used in this indirect immunofluorescence study, has been previously described (Huin et al., 2000).* **(A)** *5-day post-fecundation mouse blastocyst exhibiting an intense labeling in embryonic cell nuclei (arrowheads). Bar, 16 μm.* **(B)** *The presence of PPARβ/δ is not detected in the sclerotomes at gestation day-11. Bar, 150 μm.* **(C)** *Intestinal sections of an 11-old-day mouse embryo. Some nuclei are stained (arrows). The strong reaction in the structures (*) surrounding the intestinal loops is an artifact. Bar, 160 μm.* **(D)** *Parasagittal section of a gestation day-15 mouse embryo. At this stage of development, PPARβ/δ is ubiquitously expressed in the main tissues. Bar, 1 cm. CNS, central nervous system; DT, digestive tract; E, eye; Li, liver; Lu, lung; V, vertebrae.*

PPARs and cell life status

PPARs play pivotal roles in cell proliferation, differentiation and apoptosis (Fig. 6).

Cell proliferation

The administration of PPs to rodents is associated with an increase in replicative DNA synthesis (Marsman *et al*., 1988) leading to hepatomegaly, the extent of which is dependent upon the agent involved, the dose and duration of treatment (Hawkins *et al.,* 1987).

The mitogenic effects of PPs may be explained in part by the fact that they induce growth regulatory genes such as c-*myc,* c-*Ha-ras*, *fos, jun* and *egr-1* (Cherkaoui-Malki *et al.,* 1990; Ledwith *et al.,* 1993) or genes encoding cell cycle regulatory proteins (Chevalier and Roberts, 1999), the expression of which is important in the progression of the cell cycle to S phase. However, despite the disparate carcinogenic potential of the non-genotoxic hepatocarcinogen PP, diethylhexyl phthalate, and of the non-carcinogenic liver mitogen, 1,4-dichlorobenzene, both chemicals induce similar patterns of the immediate-early genes c-*fos*, c-*jun* and c-*myc* (Hasmall *et al.,* 1997). In addition, repeated waves of cell proliferation induced by the PP nafenopin did not result in any development of enzyme-altered pre-neoplastic hepatic foci in male Wistar rats (Columbano *et al.,* 1990).

The proliferative effects of PPs may be also due to the sustained overexpression of H2O2-generating peroxisomal fatty acyl-CoA oxidase, the first enzyme of the peroxisomal fatty acid β–oxidation system. Disproportionate increases in H2O2-generating enzymes and H2O2-degrading enzyme catalase (Reddy and Lalwani, 1983; Némali *et al.,* 1988) lead in PP-treated cells to increased oxidative stress. The latter manifests as massive accumulation of lipofuscin in exposed cells, and increased levels of 8-hydroxyguanosine adducts in their DNA. The PP-induced alterations could lead to the transformation of the treated-cells. Several data support this hypothesis: 1/ sustained overexpression of H2O2-generating peroxisomal acyl-CoA oxidase (AOX) causes transformation of mammalian cells (Chu *et al.,* 1995; Okamoto *et al.,* 1997; Dadras *et al.,* 1998; Tamatani *et al.,* 1999); 2/ African green monkey kidney cells are transformed when transfected with a full-length cDNA encoding rat urate oxidase which oxidizes uric acid to allantoin and in the process generates H2O2, and when exposed to the substrate uric acid (Chu *et al.,* 1996). However, mice with disrupted AOX gene (AOX-/-) exhibit profound spontaneous peroxisome proliferation, including development of liver tumors (Fan *et al.,* 1996, 1998). Hepatic adenomas and carcinomas develop in AOX-/- mice by 15 months of age due to sustained activation of PPARα by the unmetabolized substrates of acyl-CoA oxidase (Fan *et al.,* 1998; Yeldandi *et al.,* 2000). Effectively, the PPARα-subtype plays a pivotal role in hepatocarcinogenicity as demonstrated by recent data with PPARα-knock out mice. No hepatomegaly, hepatic peroxisome and cell proliferation are observed in PPARα-null mice treated with PPs comparatively to wild type mice (Lee *et al.,* 1995; Peters *et al*., 1997; Ward *et al.,* 1998). It is actually thought that PPARα activation by PPs leads to disregulation of hepatic acute-phase protein gene expression in rats and mice. This disregulation may indicate alterations in cytokine signaling networks regulating hepatocellular proliferation (Anderson *et al.,* 1999).

Cell differentiation

PPARs are key transcriptional factors in differentiation of several cell types. PPARα has been shown to induce differentiation of epidermal (Hanley *et al.,* 1998; Rivier *et al.,* 1998), tubular and interstitial testicular cells (Schultz *et al.,* 1999) and human hepatoblastoma Hep G2 cells (Stier *et al*., 1998). PPARβ/δ is predominantly expressed in differentiating oligodendrocytes

(Granneman *et al.,* 1998), epidermal and tracheobranchial epithelial cells (Matsuura *et al.,* 1999). Activation of this receptor finalizes sebocyte maturation (Rosenfield *et al.,* 1999). Recent data have demonstrated that activation of PPARγ increases the number of small preadipocytes in white adipose tissue (Okuno *et al.,* 1998). Thereafter, preadipocytes undergo growth arrest and subterminal differentiation into adipocytes. This is accompanied by a dramatic increase in expression of adipogenic genes containing response elements for PPARγ and for CCAAT/enhancer binding protein in their regulatory regions (Tontonoz *et al.,* 1994b; Wu *et al.,* 1999). The pivotal role of PPARγ in adipocyte differentiation is confirmed by the fact that: 1/ retroviral expression of PPARγ2 stimulates adipose differentiation of cultured fibroblasts (Tontonoz *et al.,* 1994b); 2/ the antidiabetic thiazolidinedione, a specific synthetic ligand of PPARγ, induces the trans-differentiation of myoblasts to adipoblasts (Grimaldi *et al.,* 1997); 3/ mice chimeric for wild-type and PPARγ null cells show no contribution of null cells to formation of adipose tissue whereas *in vitro* differentiation of embryonic stem cells into fat is dependent on PPARγ gene dosage (Rosen *et al.,* 1999). PPARγ plays also a major role in differentiation of other cells such as monocytes/macrophages (Tontonoz *et al.,* 1998), preputial sebocytes (Rosenfield *et al.,* 1999), epidermal cells (Rivier *et al.*, 1998) as well as colon (Brockman *et al.,* 1998; Sarraf *et al.,* 1998; Kitamura *et al.,* 1999; Lefebvre *et al.,* 1999), prostate (Kubota *et al.,* 1998) and breast (Mueller *et al.,* 1998) cancer epithelial cells. Furthermore, activation of PPARγ results in differentiation in patients with liposarcoma (Demetri *et al.,* 1999). PPARγ does not only control genes responsible for the differentiated cell phenotype, but also participates to the regulation of cell cycle withdrawal. In fact, PPARγ activation inhibits the DNA-binding and transcriptional activity of E2F/DP factors which are involved in cell growth. This decrease in E2F/DP activity is due to an increase in the phosphorylation of these proteins resulting from the down-regulation by PPARγ of protein phosphatase 2A expression (Altiok *et al*., 1997). Clearly, PPARs are "master regulators" of cell differentiation.

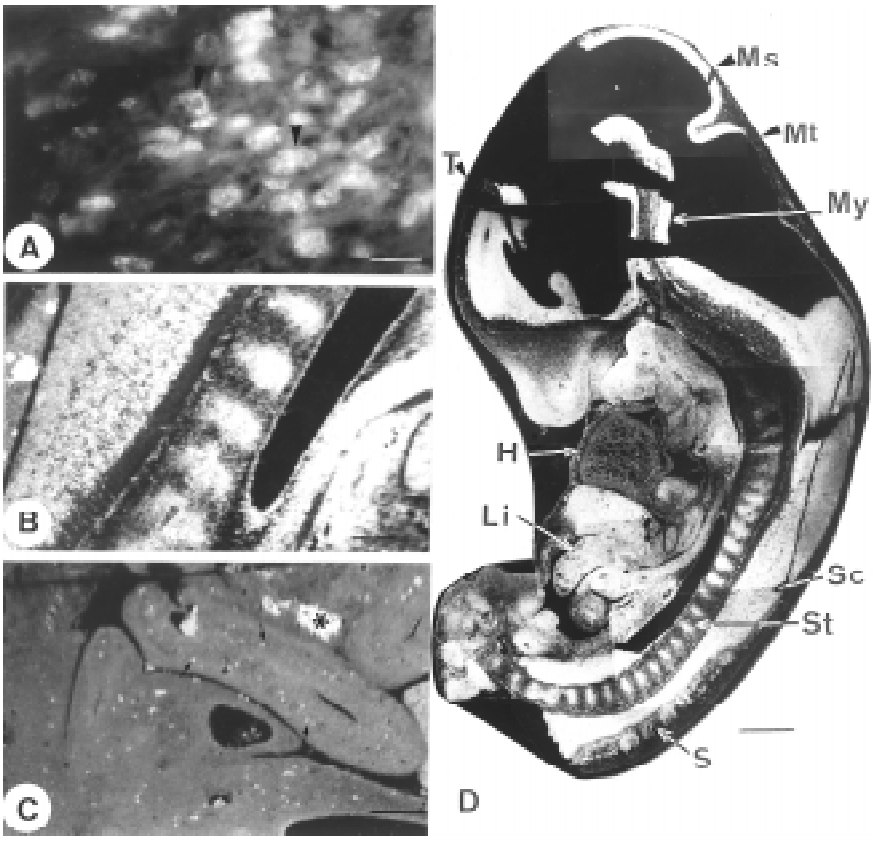

Fig. 5. Expression of PPARγ2 in the developing mouse embryo. *Using a polyclonal antibody directed against the specific NH2-terminus of PPARγ2 (Huin et al., 2000), the expression of this subtype was analyzed by indirect immunofluorescence during mouse embryogenesis.* **(A)** *The presence of PPARγ2 is faintly detected in embryonic cell nuclei (arrowheads) as soon as day-5 post-fecundation. Bar, 16 μm.* **(B)** *A diffuse staining of the sclerotomes and the spinal cord is obtained with the antibody at gestation day-11. Bar, 150 μm.* **(C)** *Two days later, PPARγ2 is faintly expressed in the intestinal epithelial cell nuclei (arrows). The strong reaction (*) is an artifact due to autofluorescence. Bar, 160 μm.* **(D)** *Parasagittal section of a gestation day-11 mouse embryo. Bar, 440 μm. H, heart; Li, liver; Ms, mesencephalon; Mt, metencephalon; My, myelencephalon; S, somite; Sc, spinal cord; St, sclerotome; T, telencephalon.*

Programmed cell death

PPs suppress apoptosis in rodent hepatoma cells and hepatocytes (Bayly *et al.,* 1993; James *et al.,* 1998b; Plant *et al.,* 1998). These data are consistent with the fact that PPs stimulate G1-arrested cells to re-enter the cell cycle and induce DNA synthesis (Chevalier and Roberts, 1999). Normal regulation of bcl-2 and bak, two proteins involved in the control of cell apoptosis, is inhibited in mouse hepatocytes by nafenopin, a potent peroxisome proliferator (Christensen *et al.,* 1998). Transient transfection of primary rat liver cells with a variant form of human PPARα which could not be activated by PPs, prevents the suppression of hepatocyte apoptosis (Roberts *et al.,* 1998). The fact that apoptosis suppression was abrogated completely even though only 30% of hepatocytes were transfected suggests the involvement of a soluble factor. Tumor necrosis factor alpha (TNFα) may be a good candidate in mediating the effects of PPs as its neutralization by specific antibodies abrogates the suppression of apoptosis by nafenopin (Rolfe *et al*., 1997).

On the other hand, apoptosis is induced by PPs in liver (Canuto *et al.,* 1998), breast (Elstner *et al.,* 1998), stomach (Takahashi *et al*., 1999) and endothelium (Bishop-Bailey and Hla, 1999) cancer cells. Consistent with PPAR role in cell differentiation, ligand activation of PPARα and PPARγ induces apoptosis of macrophages activated with TNFα/interferon gamma by negatively interfering with the anti-apoptotic nuclear factor-kappaB (NF-κB) signaling pathway (Chinetti *et al.,* 1998).

The different conclusions about the role of PPs in programmed cell death likely depend upon the PPAR subtype which they activate and/or reflect differences in cell model responses. It is presently difficult to predict whether PPAR activation could lead to beneficial or adverse effects in cancer therapy.

PPARs and human diseases

As PPARs are mainly involved in lipid metabolism and homeostasis and are activated by physiological concentrations of fatty acids, their participation in different lipid–related disorders has been recently investigated (Fig. 6).

Obesity and insulin resistance

Imbalance in energy homeostasis is responsible for different human disorders like dislipidaemia, type 2 (non-insulin-dependent) diabetes mellitus and obesity which are often causally linked to each other.

Type 2 diabetes mellitus is a heterogeneous disease resulting from a dynamic interaction between defects in insulin secretion and insulin action. In both animal and human models affected by this disorder, treatment with thiazolidinediones results in reduction of elevated plasma glucose, triglyceride and insulin concentrations, in stimulation of adipogenesis and recovery of insulin sensitivity of target tissues (Sohda *et al.*, 1990; Kletzien *et al.*, 1992; Ibrahimi *et al.*, 1994; Hallakou *et al.*, 1997; Johnson *et al.*, 1998). The antidiabetic actions of thiazolidinediones are directly mediated through binding to PPARγ and the resulting active conformation of the receptor (Ibrahimi *et al.,* 1994; Lehmann *et al.*, 1995; Berger *et al.,* 1996). Human obesity is not always associated with hyperinsulinemia. The disease is mainly characterized by accumulation of lipids in mature adipocytes and *de novo* differentiation of adipocytes from precursor cells. As outlined above, PPARγ plays a central role in the control of adipocyte gene expression and terminal differentiation. This receptor provides a molecular link between nutrition and adipogenesis as increased levels of dietary fat may activate PPARγ (Jump and Clarke, 1999). Reciprocally, a very low calorie diet induces the reduction of PPARγ mRNA level in abdominal subcutaneous adipose tissue of obese women (Bastard *et al.,* 1999).

Screening for mutations in the PPARγ gene has been performed with DNA of diabetic and/or obese patients. A genetic polymorphism corresponding to a silent C–>T substitution in the exon 6 of the PPARγ gene is associated in obese subjects with circulating leptin, a major hormonal regulator of appetite which induces satiety and increases energy expenditure (Meirhaeghe *et al.*, 1998). The conversion of proline to glutamine at position 115 of PPARγ2 positively regulates the transcriptional activity of the protein as the phosphorylation of serine at position 114 is defective. This missense mutation is found to lead to accelerated adipocyte differentiation. However, its association with obesity is nowadays discussed (Ristow *et al.*, 1998; Hamann *et al.,* 1999). In addition, a Proline12Alanine substitution in PPARγ2 has been found associated with lower body mass index and improved insulin sensitivity in diabetic and obese patients (Yen *et al.,* 1997; Beamer *et al.,* 1998; Deeb *et al.,* 1998; Koch *et al.,* 1999). Nevertheless, recent data do not support the hypothesis that this genetic variant is associated with diabetes, obesity or dislipidemia in patients with type 1 or type 2 diabetes mellitus (Mori *et al.,* 1998; Ek *et al.,* 1999; Hamann *et al.,* 1999; Mancini *et al.,* 1999; Ringel *et al.,* 1999).

It is very likely that the advances in our knowledge of the molecular mechanisms of genetic body fat disorders may lead to better prevention and treatment of obesity and other related disorders of adipose tissue in the future.

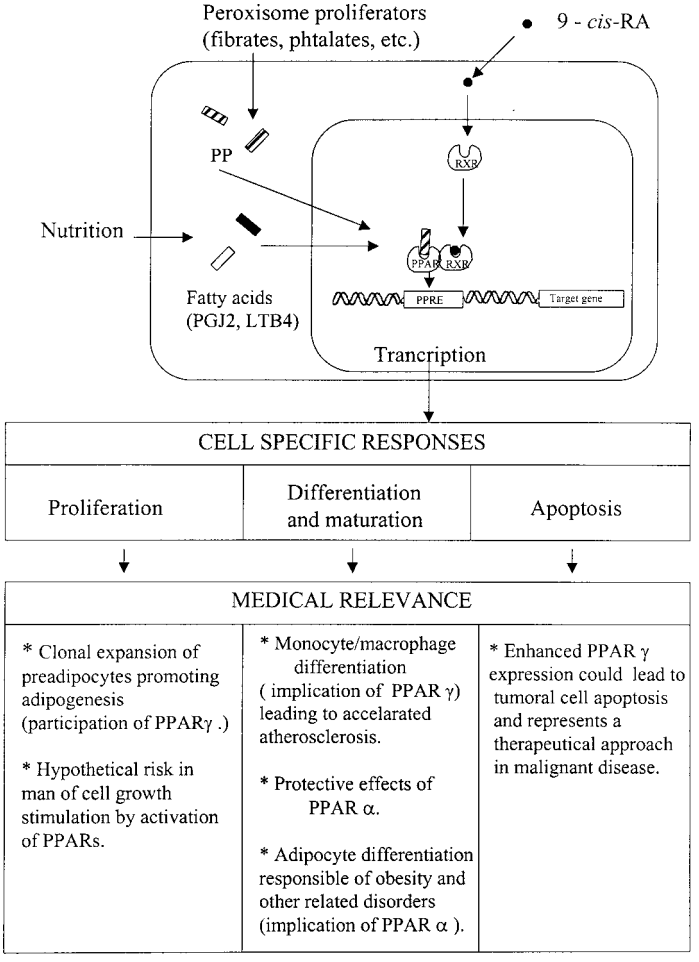

Fig. 6. Importance of PPARs in cell proliferation, differentiation and apoptosis. *After activation, PPAR and RXR form heterodimers which bind to DNA regulatory sequences of target genes through interaction with PPRE. The control by PPARs of the transcriptional activity of target genes gives rise to biological effects which may have consequences for human health. LTB4, leukotriene B4; PGJ2, prostaglandin J2; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator responsive element; 9-cis-RA, 9-cis-retinoic acid; RXR, 9-cis-retinoic acid receptor*.

Inflammation and atherosclerosis

Inflammation is a local non-specific immune response of tissues to infection and injury. The early events in inflammatory response involve the production of cytokines and chemotactic agents that initiate, coordinate and amplify the inflammatory response. LTB4 is a potent chemoattractant that is primarily involved in inflammation. It binds and activates PPARα leading to the expression of genes that terminate inflammatory processes (Devchand *et al.*, 1996). As PPARα regulates the oxidative degradation of fatty acids and their derivatives, like LTB4, it is pivotal in a feedback mechanism controlling the clearance of LTB4 in the liver and consequently the duration of an inflammatory response (Devchand *et al.*, 1996). The role of PPARα as a modulator of inflammation is also argued by the fact that the administration to aged mice of PPARα activators modulates cellular redox status, represses nuclear factor-kappaB (NF-κB) signaling and reduces inflammatory cytokine

production (Poynter and Daynes, 1998) and that PPARα-deficient mice show a prolonged response to inflammatory stimuli than wild mice (Gonzalez, 1997). The pleiotropic effects of fibrate-activated PPARα on the plasma lipid profile and vascular wall inflammation may prevent atherosclerosis development. They lead to the stimulation of hepatic uptake, esterification and oxidation of free fatty acids, the induction of lipoprotein lipase, apolipoproteins A-I and A-II expression and the inhibition of apolipoprotein C-III expression (Fruchart *et al.*, 1999). These effects contribute to the hypotriglyceridaemic action of fibrates by reducing triglyceride supply for very low-density lipoprotein (VLDL) synthesis. In addition, interleukin-1-induced production of interleukin-6 is inhibited in a dose-dependent manner in smooth-muscle cells cultured in presence of PPARα activators (Staels *et al.,* 1998). As shown by these authors, PPARα down-regulates cytokine production in these cells by interfering with different signaling pathways, inhibiting the NF-κB, activating protein 1 (AP-1) and signal transducer and activator of transcription (STAT) activities.

The precise role of PPARγ in inflammation processes remains unclear as its activation leads to both beneficial and undesirable effects. On the one hand, stimulation of PPARγ by thiazolidinedione ligands inhibits the gelatinolytic activity of the matrix metalloproteinase-9, an enzyme implicated in human atherosclerotic lesions (Marx *et al.*, 1998) and the inducible nitric oxide synthase pathway (Ricote *et al.*, 1998; Colville-Nash *et al.,* 1998) and suppresses monocyte elaboration of inflammatory cytokines (Jiang *et al.,* 1998). Thiazolidinedione ligands for PPARγ reduce colonic inflammation in a mouse model of inflammatory bowel disease (Su *et al.*, 1999). On the other hand, PPARγ plays a key role in the differentiation of monocytes to macrophage foam cells which are components of atherosclerotic plaque (Tontonoz *et al.*, 1998). Cholesterol-carrying LDL is oxidized giving rise to Ox-LDL which binds to the scavenger receptor protein CD36 on the surface of blood monocytes and is taken up by these cells. Within the cells, Ox-LDL releases cholesterol and oxidized fatty acids that are 9- and 13-hydroxyoctadecadienoic acids which are both oxidative metabolites of linoleic acid. These fatty acids activate PPARγ inducing the expression of the scavenger receptor leading to increased binding and uptake of Ox-LDL, and to the formation of foam cells (Nagy *et al.*, 1998; Tontonoz *et al.,* 1998). It is noteworthy that the anti-inflammatory action of PPARα may be explained in part by the fact that PPARα activators inhibit in a dose-dependent manner the expression of cyclo-oxygenase-2, a rate-limiting enzyme for synthesis of prostaglandins related to PGJ2 which are putative endogenous PPARγ ligands (Staels *et al.,* 1998). Further clinical investigations are necessary to provide answers to questions regarding the *in vivo* consequences of PPAR activation on tissue inflammation.

Carcinogenesis

PPs are regarded as rodent non-genotoxic carcinogens. The administration of these compounds to laboratory animals results in the development of hepatic and pancreatic cell carcinomas (Reddy and Rao, 1977) and some PPs cause Leydig cell adenomas (Biegel *et al.*, 1992). However, marked species differences are apparent in response to PPs and these rodent data do not seem relevant to humans (Gariot *et al.*, 1983; Chevalier and Roberts, 1998). The basic mechanism by which this class of chemicals induces tumor formation in rats and mice is not still understood. Several hypotheses have been advanced. As already mentioned, one is based on the development in PP-treated cells of an oxidative stress due to an imbalance in the production and degradation of peroxide hydrogen. In fact, PPs induce a drastic increase in the activity of the fatty acyl-CoA oxidase, the first peroxisomal β-oxidation enzyme, generating high levels of hydrogen peroxide meanwhile their action on catalase activity is quite low. The overproduction of reactive oxygen species leads to DNA damages and lipid peroxidation, and possibly to tumor initiation. An alternative mechanism is that PPs act rather as promoting factors of tumor formation than as initiating agents. A convergence of PPs with growth factor pathways, in particular the mitogen-activated protein kinase, has been reported by Rokos and Ledwith (1997). Suppression of apoptosis and sustained stimulation of cell growth may be sufficient to induce carcinogenesis (Marsman *et al.*, 1988; Cattley and Popp, 1989).

Recent studies have emphasized the role of PPARα in the perturbation of cell growth. PPARα null mice administered with Wy-14,643, a potent PP, do not exhibit hepatic cell proliferation (Peters *et al.*, 1997). This work clearly demonstrates that PP-induced hepatocarcinogenesis is mediated by PPARα . In addition, James *et al.* (1998a) have shown that a naturally occurring isoform of human PPARα (hPPARα-6/29) which binds to DNA but is not activated by PPs, acts as a dominant negative regulator of PPAR-mediated gene transcription. Transient transfection of hPPARα-6/29 cDNA into primary culture rat liver cells leads to the suppression of hepatocyte apoptosis by the PP nafenopin. Thus, the suppression of cell apoptosis by PPs is mediated *via* transcriptional activation of PPARα.

The anti-tumorigenic activity of nonsteroidal anti-inflammatory drugs in colorectal cancer has been recently explained through PPARβ/δ by He *et al.* (1999). The authors inferred that nonsteroidal anti-inflammatory drugs reduce the incidence of intestinal tumors by inhibiting PPARδ function and promoting apoptosis. However, additional insights are needed regarding the target genes of PPARβ/δ and their role in cell proliferation.

As the activation of PPARγ induces the differentiation of several cell types, the capacity of PPARγ agonists for the treatment of cancers has been investigated. In cultured human breast cancer cells, activation of PPARγ causes changes in gene expression associated with a more differentiated, less malignant state, and a reduction in growth rate and clonogenic capacity of the cells (Mueller *et al.,* 1998). Primary human liposarcoma cells can undergo terminal differentiation by treatment with the PPARγ pioglitazone (Tontonoz *et al.,* 1997).A clinical trial for treatment of patients with advanced liposarcoma by using troglitazone has been recently reported (Demetri *et al.,* 1999). Biopsies of tumors from these patients have revealed evidence of terminal adipocytic differentiation and reduction in expression of Ki-67, a marker of cell proliferation. Taking together these data suggest that activators of PPARγ may represent chemotherapeutic agents in the treatment of these human cancers. On the other hand, it has been recently suggested that activation of PPARγ can both induce and inhibit the development of colonic tumors. PPARγ gene and protein expression is elevated in rodent colon tumors as well as in selected human colon cancer cell lines (Dubois *et al.*, 1998). Activation of PPARγ with synthetic ligands increases the frequency and size of colon tumors in mice predisposed to intestinal neoplasia (Lefebvre *et al.*, 1998; Saez *et al.,* 1998). In contrast to these data dealing with the

participation of PPARγ in colon polyp and tumor formation, recent papers demonstrate that in humans activation of PPARγ reduces colonic inflammation (Su *et al.*, 1999), increases the differentiated status of colonic cancer cells and slows their growth (Brockman *et al.*, 1998; Sarraf *et al.*, 1998; Kitamura *et al.*, 1999). Furthermore, loss-of-function mutations in PPARγ are often associated with human colon carcinomas (Sarraf *et al.*, 1999). These conflicting results which may be due to species differences, indicate the need for a better understanding of the role of PPARγ in the colonic physiology and cancer development.

Conclusions

As can be seen from this review, PPARs are important during development. These receptors are temporally and spatially expressed throughout development and adult life. Of particular interest is the fact that PPARs and RXRs share some common spectra of expression during murine development. This leads to the proposal that PPAR/RXR heterodimers are involved in the developmental program. This proposal is also supported by the identification of RXR target genes involved in lipid and fatty acid metabolism (Mangelsdorf *et al.,* 1994). This argues in favor of a synergistic action of nutrients and retinoids in the different aspects of development, from implantation of the embryo to organogenesis and differentiation as well as in adult physiology and metabolism.

PPARs are also key control elements in cell proliferation, differentiation and apoptosis. Although most genes known to be regulated by these receptors are involved in fatty acid metabolism and homeostasis, there are several PP-responsive genes with a link to cell life status. In this process, PPs induce the expression of proto-oncogenes or genes encoding growth factors and their receptors explaining their convergence with growth factor pathways (Vanden Heuvel, 1999). But so far, a direct action of PPARs in this induction has not been reported. In fact, our knowledge is incomplete and more research is needed about the functions of PPARs, especially PPARβ/δ. It is very likely that in the next future, new genes with PPREs in their regulatory sequences will be discovered providing new insights about the action of PPARs in cell cycle control. Since there are marked differences among cell types in their responsiveness to PPs, improvements in human health may reside in the development of new potential and specific ligands for each PPAR taking advantage of our understanding of PPAR functions.

Summary

The past several years have seen an increasing interest in the peroxisome proliferator-activated receptors (PPARs). These transcriptional factors belong to the superfamily of the steroid/thyroid/retinoid receptors. They are activated by fatty acids or their metabolites as well as by different xenobiotic peroxisome proliferators. These receptors are expressed in both the embryo and the adult organism. They have been implicated in cell proliferation, differentiation and apoptosis. In this review, we will attempt to point out some of the more salient features of this expression pattern during development and the different steps of cell life. The current understanding of how PPARs are involved in some human diseases will also be described.

Acknowledgments

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