Annu. Rev. Pharmacol. Toxicol. 2000. 40:491–518

CENTRAL ROLE OF PEROXISOME PROLIFERATOR–ACTIVATED RECEPTORS IN THE ACTIONS OF PEROXISOME PROLIFERATORS

J. Christopher Corton, Steven P. Anderson[1], and Anja Stauber
Chemical Industry Institute of Toxicology, 6 Davis Drive, Research Triangle Park, North Carolina 27709–2137; e-mail: corton@ciit.org, stauber@ciit.org

Key Words hepatocarcinogenesis, inflammation, growth suppression

■ **Abstract** Peroxisome proliferators (PPs) are a large class of structurally dissimilar chemicals that have diverse effects in rodents and humans. Most, if not all, of the diverse effects of PPs are mediated by three members of the nuclear receptor superfamily called peroxisome proliferator-activated receptors (PPARs). In this review, we define the molecular mechanisms of PPs, including PPAR binding specificity, alteration of gene expression through binding to DNA response elements, and cross talk with other signaling pathways. We discuss the roles of PPARs in growth promotion in rodent hepatocarcinogenesis and potential therapeutic effects, including suppression of cancer growth and inflammation.

INTRODUCTION

Peroxisomes are subcellular organelles that are found in most animal cells and that perform diverse metabolic functions, including H_2O_2-derived respiration, β-oxidation of fatty acids, and cholesterol metabolism (1). Peroxisome proliferators (PPs) are a large class of structurally dissimilar industrial and pharmaceutical chemicals that were originally identified as inducers of both the size and the number of peroxisomes in rat and mouse livers or hepatocytes in vitro after exposure. Rodent exposure to PPs leads to a stereotypical orchestration of adaptations consisting of hepatocellular hypertrophy and hyperplasia, and to transcriptional induction of fatty acid–metabolizing enzymes regulated in parallel with peroxisome proliferation (1). Chronic exposure to many PPs causes an increased incidence of liver tumors in male and female mice and rats (2).

[1]Present Address: Strategic Toxicologic Sciences, Glaxo Wellcome Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709

0362–1642/00/0415–0491$14.00

Recent research points toward a pivotal role for a subset of nuclear receptor superfamily members, called peroxisome proliferator–activated receptors (PPARs), in mediating many, or all, of the adaptive consequences of PP exposure (3). PPs may activate PPARs by binding directly to the receptor, or possibly by perturbing lipid metabolism to generate PPAR ligands. Upon activation, PPAR regulates the expression of genes involved in lipid metabolism and peroxisome proliferation, as well as genes involved in cell growth. Other factors, both positive and negative, influence the ability of PPARs to modulate gene expression.

In this review, we describe the molecular mode of action of PPAR transcription factors, including ligand binding, interaction with specific DNA response elements, transcriptional activation, and cross talk with other signaling pathways. We discuss the evidence that suggests that PPARs play a central role in mediating rodent hepatocarcinogenesis. Lastly, we discuss evidence that has recently emerged on the potential therapeutic actions of PPs, including suppression of cancer cell growth and inflammation. Readers are referred to a number of excellent reviews focusing more specifically on the role of PPARs in hyperlipidemia (4), type II diabetes (5), adipocyte differentiation (6), and atherosclerosis (7, 8).

STRUCTURE AND FUNCTION OF PPAR ISOFORMS

The rapid and coordinate induction of liver cell hyperplasia, peroxisome proliferation, and increases in lipid-metabolizing enzymes by PPs gave an early indication that a receptor-mediated mechanism was involved (9). A member of the nuclear receptor superfamily that was transcriptionally activated by PPs was cloned from mouse liver in 1990 and was named the peroxisome proliferator–activated receptor (PPAR) (10). Like other members of the vertebrate steroid-nuclear receptor superfamily, PPARs exist in distinct isoforms encoded by separate genes. There are three known PPAR isoforms: PPARα, PPARδ (also known as NUC1 and PPARβ), and PPARγ (11).

Similar to other nuclear receptors, PPAR proteins exhibit a generic organization consisting of a number of functional domains: A/B, C, D, and E/F (reviewed in 12). The A/B region encodes a ligand-independent transcriptional activation domain (activation function-1) that is active in some cell types. The C domain encodes the highly conserved DNA binding domain consisting of two zinc finger DNA binding motifs. This domain targets the receptor to specific DNA sequences in responsive genes called peroxisome proliferator response elements (PPREs). The ligand binding domain (LBD), or E domain of PPARs, is responsible for ligand-binding and converting PPARs to an active form that binds DNA and modulates gene expression. In addition, the E region is also important in dimerization, nuclear localization, and association with modulators of transcription, such as coactivators and corepressors, through interaction with a transactivation domain [activation function-2 (AF-2)] located within the C-terminal α-helix. PPARs lack a C-terminal extension (the F domain) found in other nuclear recep-

tors that may modulate transactivation. The D region encodes a flexible hinge region, thought to allow independent movement of the LBD relative to the DNA binding domain.

PPAR isoforms perform different physiological functions, based on their divergent patterns of tissue-specific expression, different ligand-binding specificities, and divergent physiological consequences when activated (Table 1). PPARα regulates fatty acid metabolism and is highly expressed in liver, kidney, and intestine. PPARα also down-regulates inflammatory responses (13, 14). PPARβ/δ is ubiquitously expressed, but its physiological function is yet to be fully defined. By using a specific agonist, PPARβ/δ has been shown to be involved in embryo implantation and decidualization in the mouse (15). PPARγ exists in two distinct isoforms, designated PPARγ1 and PPARγ2, that have different tissue distributions and functions. Expression of these isoforms, differing only in their N-terminal 30 amino acids (γ2 has 30 extra amino acids), is driven from the same gene by alternative promoter usage and splicing (16). PPARγ1 is found in liver and to a lesser extent in other organs, including adipose tissue. PPARγ2 is expressed exclusively in adipose tissue and is a potent regulator of adipocyte differentiation. When PPARγ2 is expressed in fibroblasts, adipocyte genes are activated, leading to conversion of fibroblasts to adipocytes (reviewed in 6, 17). Activation of PPARγ inhibits angiogenesis (18) and inflammatory processes, which are involved in a number of disease states (5). Thiazolidinedione drug activation of PPARγ induces the antidiabetic effects of this important class of compounds (5).

Recent evidence demonstrates that PPARs modulate gene expression in a manner similar to that of other nuclear receptors (Figure 1). Many important clues to the mechanism of PPAR activation have come from resolving the crystal structures of the LBDs of the human PPARγ (19, 20) and human PPARβ/δ (21) determined both with and without ligands. The PPAR ligand binding pocket, encompassing ~1300 $Å^3$, is unusual in that it is two to three times larger than pockets in other nuclear receptors (22). This difference in size allows these receptors to accommodate a host of structurally diverse chemicals (see below). In the absence of ligand activation, PPAR AF-2 helix is positioned away from the ligand binding pocket (19, 20). Ligand binding induces a conformational change in the

TABLE 1 Properties of rodent isoforms of the peroxisome proliferator-activated receptor

	Tissue distribution[a]					
Isoform	**Liver**	**Kidney**	**Intestine**	**Spleen**	**Fat**	**Physiological role**
α	++++	++	++++	+	−	Lipid metabolism, regulation of inflammation
β/δ	++	++	+++	++	−	Embryo implantation
γ	−	+/−	++	+++	++++	Adipocyte differentiation, regulation of inflammation

[a]Tissue distribution is based on in situ hybridization of rat tissue (90).

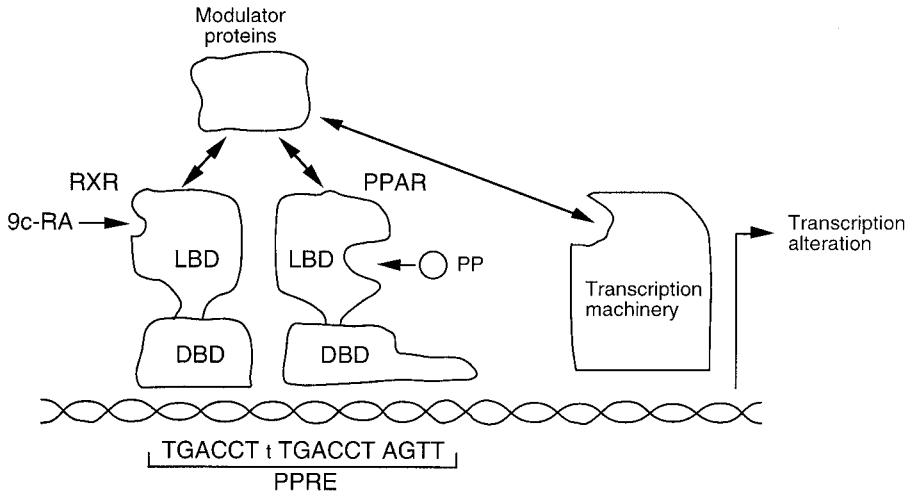

Figure 1 Modulation of gene transcription by a peroxisome proliferator–activated receptor (PPAR). Ligand binding of the peroxisome proliferator (PP) leads to PPAR activation and heterodimerization with the retinoid X receptor (RXR), the receptor for 9-*cis*-retinoic acid (9c-RA). The PPAR-RXR heterodimer, through their DNA-binding domains (DBD), binds to the consensus sequence 5'-TGACCT T TGACCT AGTT-3' (or variant), with PPAR occupying the 3' position. Interactions between the PPAR-RXR heterodimer, modulator proteins, and the transcription machinery affect transcription initiation and mRNA abundance. LBD, ligand-binding domain; PPRE, peroxisome proliferator response element.

LBD, resulting in the AF-2 helix swinging shut behind the ligand, in what has been termed the mouse trap model (19). Critical interactions between the acidic group on the ligand and specific amino acids on the AF-2 helix are required for ligand binding and associated transcriptional activation. The pivotal role of AF-2 helix amino acids in the transactivation function of PPARs and other nuclear receptors had been previously demonstrated (reviewed in 23). The ligand-induced conformation of the LBD, including the AF-2 helix across the opening of the ligand binding pocket, creates the proper orientation for binding to a coactivator protein called the steroid receptor coactivator-1 (SRC-1). A number of proteins, including coactivators, encode one or more short α-helices, called nuclear receptor boxes, which are important in nuclear receptor interaction and transcriptional activation. A nuclear receptor box from SRC-1 associates with the PPARδ LBD between a glutamic acid on the AF-2 helix and a lysine on helix 3. This arrangement of the nuclear receptor box helix held between PPAR LBD amino acids has been called the charged clamp (19). In addition to interaction with transcriptional modulators, PP binding to PPARs leads to dimerization with the retinoid X receptor (RXR), the receptor for 9-*cis* retinoic acid. The PPAR-RXR heterodimer binds

to the consensus sequence 5'-TGACCT T TGACCT AGTT-3' (or variant), with PPARs occupying the 3' position. Interaction between the PPAR-RXR heterodimer and other factors that modulate the PP-induced activation, such as coactivators, the transcription machinery, or both, leads to either increases or decreases in transcription of target genes that contain PPREs. Transcription may also be modulated by phosphorylation of the A/B domains of PPARα (24) and -γ (25) through a mitogen-activated protein kinase-dependent pathway (23).

PPAR LIGANDS

Exogenous Ligands

PPs are unique, compared with the structurally restricted ligands that interact with other nuclear receptors, in that they are structurally diverse. PPs do have similar structural requirements for interacting with and activating PPARs in vitro and for eliciting biological effects in humans or animals. Most PPs are amphipathic molecules containing a hydrophobic backbone (aliphatic or aromatic) linked to an acidic function. This acidic function is essential for ligand activity and typically consists of a carboxyl group present in the parent compound or a group that may be converted metabolically to a carboxyl group. PPs resemble endogenous lipid activators of PPARs that also require an acidic function linked to an aliphatic backbone for activity.

Several PPs have been shown to bind to PPARs and to act as exogenous PPAR activators in cell transactivation assays (Table 2). These include hypolipidemic drugs such as clofibrate and gemfibrozil and the experimental drug WY-14,643 (26, 27), leukotriene D4 receptor antagonists (28–30), and industrial compounds (10, 31). Several of these PPs have been shown to preferentially activate the PPARα isoform, which suggests that some PPs may be PPARα-selective ligands (26, 27). There is a good correlation between the ability of a PP to bind to and/or activate PPARα and the potency of the PP as an inducer of hepatocarcinogenesis. For example, one of the strongest inducers of hepatocarcinogenesis is WY-14,643, which binds strongly to PPARα and activates PPARα to high levels. In contrast, bezafibrate is a relatively weak hepatocarcinogen and binds and activates PPARα weakly (32). Taken together, these studies strongly suggest that many hepatocarcinogenic PPs are ligands of the PPARα isoform, and they support the hypothesis that PPARα is the major cellular target of hepatocarcinogenic PPs in the liver.

Antidiabetic thiazolidinediones enhance adipocyte differentiation, a process shown to be mediated by PPARγ (6). One such compound, BRL49653, specifically binds and activates PPARγ in transactivation assays (27, 33). Other thiazolidinedione and non-thiazolidinedione chemicals have more recently been identified as PPARγ-specific ligands. The non-thiazolidinedione L-764406 binds to PPARγ with high affinity, forms a covalent linkage with Cys^{313} in helix 3 of

TABLE 2 Chemical activators of peroxisome proliferator-activated receptors (PPARs)[a]

	PPAR		
Peroxisome proliferator compounds	α	**β/δ**	**γ**
Hypolipidemic drugs			
WY-14,643[b]	+++[c]	+	++
Clofibrate[a]	++[c]	−	+
Ciprofibrate[b]	++[c]	−	+
Gemfibrozil[a]	++[c]	−	+
Nafenopin[b]	++[c]	ND	ND
GW2331	+++[c]	+/−	++[c]
Bezafibrate[b]	+[c]	+++[c]	−
Miscellaneous peroxisome proliferators			
Phthalate ester			
Monoethylhexyl phthalate[b,d]	+++	+	++
Organic Solvent			
Trichloroacetic acid[a]	+	ND	ND
Synthetic arachidonic acid			
ETYA	+++[c]	−	+/−
Leukotriene B4 antagonists			
MK-571	++	ND	ND
LY-171883	++[c]	ND	ND
Antidiabetic thiazolidinediones			
BRL-49653	−	−	+++[c]
Pioglitazone	−	−	++[c]
Ciglitazone	−	−	++[c]
Englitazone	−	−	+[c]
KRP-297	++[c]	−	++[c]
MCC-555	ND	ND	++
Nonsteroidal ant-inflammatory drugs			
Indomethacin	+	−	+++[c]
Ibuprofen	+	−	+[c]
Fenoprofen	++	−	+[c]
Piroxicam	−	−	+
GW2433	−	+++	−
GW0072	−	−	+
L-764406	−	−	+++
L-165041	−	+++	+

[a] +,Activator of this isoform in a transactivation assay; −, not an activator of this isoform in a transactivation assy; +/−, conflicting reports in the literature about this compound being an activator in a transactivation assay; ND, not determined for this compound.

[b] This compound has been shown to cause hepatocarcinogenesis in rats and mice (see Reference 3).

[c] This compound has been determined to be a ligand for the PPAR isoform.

[d] This compound is believed to be the proximate carcinogen of di(2-ethylhexyl)phthalate (see text and Reference 3).

the LBD, and acts as a partial agonist (25% of highest activity obtained with a thiazolidinedione) in transactivation of PPARγ and induction of the adipogenic gene program (34). Another non-thiazolidinedione has been identified that is similar to classical receptor antagonists. GW0072 binds to PPARγ with high affinity and is a weak agonist, but it potently inhibits the ability of a thiazolidinedione to activate PPARγ and the associated adipocyte differentiation program (35). Interference of thiazolidinedione activation by GW0072 occurs through a unique mode of binding. The bound receptor adopts a conformation similar to the unliganded apo-receptor in which the carboxylic acid of GW0072 is oriented away from the AF-2 helix, preventing the charged clamp to be stabilized through direct interactions with the ligand. GW0072 may be the prototype of ligands with unique biological activities mechanistically distinct from conventional agonists and antagonists. A number of other non-thiazolidinedione chemicals have been identified that interact with both PPARγ and PPARδ (36).

Several nonsteroidal anti-inflammatory drugs (NSAIDS), such as indomethacin and ibuprofen, that are cyclooxygenase (COX) inhibitors have been shown to activate and bind PPARγ and promote adipocyte differentiation at concentrations incrementally higher than those required to inhibit COX (37). Certain NSAIDS also activate PPARα (37).

Endogenous Ligands

A summary of the large number of endogenous PPAR ligands is shown in Table 3. Diverse fatty acids bind and activate all three PPAR isoforms to varying degrees (28, 38–40). The ligand binding pocket accommodates a diverse array of saturated, monounsaturated, and polyunsaturated fatty acids. Fatty acids with chain lengths under 16 and over 22 carbons activate the PPARs weakly, if at all. PPARα is the most promiscuous of the PPAR family members, exhibiting strong binding affinity for both saturated and unsaturated fatty acids. Although more selective, PPARδ will also bind diverse fatty acids, but with a lower affinity than PPARα. PPARγ is the most selective receptor because it binds primarily to polyunsaturated fatty acids (21).

An intriguing observation, which potentially implicates PPAR activation with a number of diverse functions, including tumor promotion and anti-inflammatory effects, is the recent demonstration that several leukotriene and prostaglandin (PG) eicosanoid metabolites bind and activate PPARs (28, 33, 40, 41). PGs play an important role in cancer development and progression (reviewed in 42). Decreased levels of PGs, perhaps a result of increased catabolism (43), are seen in rats and rat hepatocyte cultures treated with PPs (44). PG can increase the transcription of genes encoding enzymes involved in their own catalysis through fatty acid β- and ω-oxidation pathways, which suggests that PPARs are involved in an autoregulatory loop in lipid homeostasis (10). The inflammatory mediator leukotriene B_4 (LTB4) binds and activates PPARα, and this reportedly decreased the duration of the inflammatory response by increasing LTB4 degradation (13).

TABLE 3 Endogenous activators of peroxisome proliferator-activated receptors[a]

Activator	α	β/δ	γ
Saturated fatty acids			
Palmitic (16:0)	+++[b]	++	−
Stearic (18:0)	+++	++	−
Monounsaturated fatty acids			
Palmitoleic (16:1)	+++[b]	+	++[b]
Oleic (18:1)	+++[b]	++	++
Elaidic (20:1)	++	−	+
Polyunsaturated fatty acids			
Linoleic (18:2, n-6)	+++[b]	++	+
α-Linoleic (18:3, n-3)	+++	+	++
γ-Linoleic (18:3, n-6)	+++	+++	++
Dihomo-γ-linoleic (20:3, n-6)	+++	++	+++
Arachidonic (20:4, n-3)	+++[b]	++[b]	+++
Eicosapentaenoic (22:5, n-3)	+++[b]	++	+++
Docosahexaenoic (22:6, n-3)	++[b]	++	+/−
Eicosanoids			
PGA1	+	++	+
PGA2	+	+	+
PGD1	++	+	++
PGD2	++	+	++
PGJ2	+	+/−	+++
15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2	+	+/−	+++[b]
8(S)-HETE	+++[b]	−	−
8-HEPE	++[b]	−	−
LTB4	+/−	ND	ND
9-HODE	ND	ND	+++[b]
13-HODE	ND	ND	+++[b]

[a] +,Activator of this isoform in a transactivation assay; −, not an activator of this isoform in a transactivation assy; +/−, conflicting reports in the literature about this compound being an activator in a transactivation assay; ND, not determined for this compound. LT, leukotriene; PG, prostaglandins; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; HODE, hydroxyoctadecadiemoic acid.
[b]This compound has been determined to be a ligand for the peroxisome proliferator-activated receptor isoform.

The identification of eicosanoid metabolites as PPAR ligands should clarify the metabolic and regulatory roles of PPAR-dependent pathways in hepatocarcinogenesis and suppression of inflammation.

Lastly, oxidized low-density lipoproteins thought to play a central role in the pathogenesis of atherosclerosis are partly composed of 9-hydroxyoctadecadienoic

acid (HODE) and 13-HODE, which bind and activate PPARγ and regulate some of the events important in foam cell formation (45, 46).

PEROXISOME PROLIFERATOR RESPONSE ELEMENTS

PP binding induces a conformational change in PPARs that enables binding to specific DNA sequences upstream of the transcription initiation site called peroxisome proliferator response elements (PPREs). In most cases this leads to transcriptional activation of the target gene. The first PPRE sequences were identified by promoter analysis of the PP-responsive gene, acyl-coenzyme A oxidase (ACO) (47, 48). Through similar analysis of the promoters of several other PP-responsive genes, a PPRE sequence motif was defined as two direct TG(A/T)CCT repeats, known as half-sites, separated by a single nucleotide and thus called a direct repeat one (DR1). PPREs located at variable distances upstream of the transcription initiation site have been identified in other genes known to be activated by PPs, including genes encoding peroxisomal, microsomal, mitochondrial, nuclear, and cytosolic or extracellular proteins (reviewed in 3). Comparison of PPRE sequences with the DNA-binding motifs of other nuclear receptors, including the thyroid hormone receptor (TR), the retinoic acid receptor (RAR), retinoid X receptor (RXR), and vitamin D_3 receptor (VDR), revealed that these receptors all recognize the same half-site sequence motifs (TGACCT). Like PPARs, these receptors bind to direct repeats as heterodimers with a common partner, RXR. The relative spacing and orientation of the half-site motifs determine which nuclear receptor-RXR heterodimer binds to the response element. The RXR heterodimers with RAR, TR, VDR, RAR, and PPAR recognize direct repeats separated by five, four, three, two, or one nucleotide, respectively. In addition to the DR1 sequence, PPAR-RXR heterodimers have also been shown to bind to and activate at a direct repeat element separated by two nucleotides in the promoter of the human rev-erb α gene (49) and at estrogen response elements (50).

A number of studies point to the importance of the sequences flanking the PPREs for maintaining the optimal conformation of the PPAR-RXR heterodimer on the PPREs. In rabbit cytochrome P450 4A6 PPREs, six nucleotides adjacent to the DR1 element are necessary for both optimal receptor binding and receptor gene activation (51). Likewise, in human ACO PPREs, the flanking sequences protect the PPAR/RXR heterodimer from protease digestion (52). Although these extended sequences are not essential for PPAR/RXR binding to the PPREs, they may allow the optimal positioning of the PPAR-RXR heterodimer for interactions with the transcriptional machinery, resulting in either activation or repression. In addition, these flanking sequences may provide an extra level of specificity to different nuclear receptors that recognize the DR1 element (53).

Not all of the PPREs in responsive genes act to mediate increases in transcription. A growing number of genes including transthyretin, some apolipoprotein genes, transferrin, and hepatocyte nuclear factor-4 possess DR1-like motifs but

are negatively regulated by PPs through PPARs (reviewed in 3). As discussed below, PPARs may negatively regulate some genes through DR1-like elements by competing for binding to the DR1 with other nuclear receptors that constitutively activate expression.

CONVERGENCE OF PEROXISOME PROLIFERATOR AND NUCLEAR RECEPTOR SIGNALING

Several studies have demonstrated cross talk between the PPAR signaling pathway and other nuclear receptor pathways. This convergence and interaction of different pathways can occur at multiple levels, including competition between PPARs and other nuclear receptors for (*a*) a common heterodimerization partner or (*b*) binding to the same DNA response element (Figure 2). Cross talk between PPs and other signaling pathways could explain some of the diverse biological effects of PP exposure.

One type of cross talk results from the heterodimerization of PPARs and RXR. Transactivation assays have demonstrated that PPAR-RXR heterodimers interacting at a PPRE can respond to either PPs or the RXR ligand, 9-*cis*-retinoic acid, by activation of the reporter gene. In the presence of both activators, synergistic activation of reporter gene expression occurs (54). In the absence of RXRs, PPARs cannot bind to the PPREs and activate transcription (54–56). A number of retinoids have been shown to activate the PPAR-RXR heterodimer through binding RXRs (57–59). In intact animals or cell cultures, exposure to these retinoids results in peroxisome proliferation and increases in β-oxidation enzymes

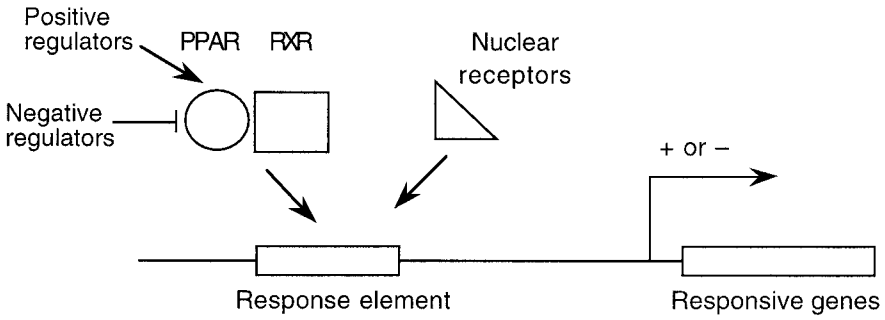

Figure 2 Mechanisms of cross talk with peroxisome proliferator–activated receptors (PPARs). Two general mechanisms of cross talk with PPAR exist. A large number of proteins interact with PPARs and may modulate the ability of PPARs to interact with a retinoid X receptor (RXR), bind a peroxisome proliferator response element, and increase transcription initiation. In addition, PPARs compete with other nuclear receptors for response element binding. The outcome is influenced by availability of the competing receptors and also by relative binding affinities for a particular response element.

(59). It is interesting to note that activation of the PPAR-RXR heterodimers through RXRs is not equivalent to activation through PPARs, as some retinoids elicit a unique set of responses not shared with PPs (59). These studies demonstrate how certain retinoids can act as PPs indirectly, through activation of the heterodimer via the back door (RXRs).

The fact that the RXR is a heterodimerization partner shared by PPARs and other nuclear receptors provides a level of cross talk through competition of available RXRs. The consequence of this competition is well-defined for PPARs and TRs. In transactivation assays, overexpression of PPARs in the presence of limiting amounts of RXRs prevents TR-RXR heterodimers from forming, resulting in inhibition of the expression of TR-regulated genes. On the other hand, an excess of TR prevents PPAR-RXR heterodimerization, resulting in repression of PPAR-regulated genes (60–63). Competition between PPARs and other nuclear receptors for RXR binding is also possible but has not been reported.

Modulation of PPAR activation by PPAR-binding proteins may also occur by either direct competition with RXRs for PPAR binding or by interaction with PPAR-RXR heterodimers on the PPREs. The large number of proteins now known to interact with PPARs is shown in Table 4. They belong to a number of predictable categories of proteins, including nuclear receptors, coactivators, and corepressors. A number of proteins have been isolated that heterodimerize with PPARs, preventing PPARs from interacting with RXRs and activating at PPRE-containing genes. These proteins include (*a*) a member of the nuclear receptor family that lacks a DNA-binding domain called SHP (64), (*b*) the nuclear receptor LXRα (65), and (*c*) deoxyuridine triphosphatase (66). The product of the nuclear oncogene *c-jun* was also shown to inhibit the ability of PPARs to activate a PPRE-linked gene, possibly through a direct interaction (67). Conversely, PPARs were able to inhibit the ability of Jun protein to activate transcription of the glutathione S-transferase–placental gene (67). The relevance of these interactions for repression of PPAR activation in mammals is not known. It is possible that these PPAR-containing heterodimers have new DNA binding targets and transcriptional properties.

Like many other nuclear receptors, PPARs are also regulated by factors referred to as coactivators and corepressors. Coactivators or corepressors act as bridging proteins between nuclear receptors and the transcriptional machinery and enhance or decrease transcriptional activation, respectively. A number of PPAR-interactive proteins have been isolated that enhance PPAR-mediated activation of reporter genes in vitro. These coactivators include p300/CBP (68), tuberous sclerosis 2 (69), PPAR binding protein (70), PGC-1 (71), PGC-2 (72), Ara70 (73), and the steroid receptor coactivator-1 (SRC-1) (74, 75). SRC-1 makes an insignificant contribution to PPARα activation in the liver because PPARα-dependent activation of PP-inducible liver genes was not altered in a mouse strain lacking a functional SRC-1 gene (76). Given the large number of coactivators that can interact with PPARs, it is likely that coactivators carry out functionally redundant roles with PPARs and other transcription factors. Although not a coac-

TABLE 4 Peroxisome proliferator-activated receptor (PPAR)-interactive proteins[a]

Protein	Interacts with PPAR			Functional consequence of interaction
	α	β/δ	γ	
Nuclear receptors				
RXR	+	+	+	Increased activation
LXRα	+	ND	ND	Inhibition of PPAR-RXR
SHP	+	ND	ND	Unknown
Coactivators				
SRC-1/TIF2	+	ND	+	
P300/CBP	ND	ND	+	
PBP	+	ND	+	
PGC-1	ND	ND	+	
PGC-2	−	−	+	
ARA70	ND	ND	+	
TSC2	ND[b]	ND	ND	
Corepressors				
N-CoR/RIP13	+	ND	+/−	Inhibition of activation
SMRT/TRAC	+	ND	+	
RIP140	+	ND	+	
Miscellaneous proteins				
c-Jun	+	ND	ND	Inhibition of activation
dUTPase	+	+	+	Prevents PPAR-RXR heterodimerization
HMG-CoA synthase	+	ND	ND	Increased activation
NRBF-1	+	ND	ND	Unknown

[a] +, Physical interaction with indicated PPAR isoform; −, no interaction; +/−, conflicting reports in the literature about interaction; ND, not determined for this isoform. RXR, retinoid X receptor; HMG, 3-hydroxy-3-methylglutaryl.
[b] Increased transactivation of designated isoform.

tivator, the 3-hydroxy-3-methylglutaryl–coenzyme A synthase protein may positively regulate its own gene by interacting with PPARα (77).

Two corepressors for RARs and TRs have been characterized that interact with unliganded receptors to keep them in a repressed state. These corepressors are termed nuclear receptor corepressor or RXR–interacting protein 13 (N-COR/RIP13) and silencing mediator for retinoid and thyroid hormone receptors or TR-associating cofactor (SMRT/TRAC). By using the two-hybrid system, interactions were detected between N-COR/RIP13 or SMRT/TRAC and PPARα (78, 79), and N-COR/RIP13 was shown to suppress PPARα-RXR transcription at a PPRE in a human kidney cell line (80). PPARγ was shown to interact with both N-COR/RIP13 and SMRT/TRAC in solution (81), although interaction between PPARγ and N-COR/RIP13 in yeast was not detected. In contrast to PPARα, these corepressors lacked the ability to interact with PPARγ-RXR heterodimers bound to

the PPRE or to repress transcription (81). Although originally identified as a coactivator, RIP140 was found to down-regulate PPARα coactivation mediated by SRC-1 possibly by competition with SRC-1 for binding to the PPARα AF-2 domain (82, 83).

In addition to competition for common heterodimerization partners, a second form of cross talk between PPARs and other nuclear receptor pathways involves competition for DNA binding at common response elements. Competition for response element binding by multiple nuclear receptor complexes can have reciprocal effects on gene expression. On the one hand, competing nuclear receptor complexes could interfere with PPAR-RXR binding to a PPRE. On the other hand, PPAR-RXR can bind to other DNA response elements and interfere with signaling by other nuclear receptors. Several nuclear receptors other than PPARs can bind to PPRE-like DR1 elements, resulting in either transcriptional repression or activation. TRα homodimers bind to PPREs, resulting in thyroid hormone-independent activation of acyl-coenzyme A oxidase (61, 63). Heterodimers of RAR-RXR have also been shown to activate at a PPRE (84). COUP-TFI (85) and COUP-TFII (also called ARP-1) (86) block PPAR action by binding PPREs when cotransfected into cell lines. Heterodimers of TR-RXR (87) and homodimers of another nuclear receptor, TAK1 (88), also inhibit PP signaling in this manner.

There are two well-characterized examples where PPAR-RXR heterodimers bind to response elements recognized by other nuclear receptors and interfere with nuclear receptor signaling. First, PPAR-RXR heterodimers were shown to compete with hepatocyte nuclear factor-4 (HNF-4) homodimers for binding to DR1 elements, resulting in decreases in transcription of apolipoprotein clII and transferrin genes (89, 90). HNF-4 binding and activation is necessary for constitutive expression of some liver-specific genes, and binding of PPAR-RXR to these HNF-4–dependent DR1 elements blocks gene expression by an unknown mechanism. It is possible that the sequences flanking the DR1 (discussed above) necessary for correctly orienting the PPAR-RXR heterodimers on the PPREs for optimal activation are lacking in these DR1 elements. Additionally, the antagonism is believed to be partially due to PPAR-RXR down-regulation of the HNF-4 gene itself, by decreasing HNF-4 autoactivation through a DR1 in the HNF-4 promoter region (90), although down-regulation of HNF-4 gene by fibrates was not observed in another study (91). Because there are many liver-specific genes whose expression appears to be dependent on HNF-4, suppression by PPAR activation could dramatically alter liver-specific functions.

In the second example, PPAR-RXR heterodimers were shown to compete with the estrogen receptor (ER) homodimer for binding to estrogen response elements (50). By using a reporter gene construct with an artificial promoter consisting of an estrogen response element linked to a basal promoter, PPAR-RXR expression was shown to activate gene expression. On a more natural promoter that contained an estrogen response element from an ER-dependent gene, PPAR-RXR heterodimers prevented ER from activating reporter gene expression (50). The ability

of PPAR-RXR to prevent ER-mediated gene expression may account for estrogen insensitivity in cells that express both ERs and PPARs (92).

PEROXISOME PROLIFERATOR-INDUCED HEPATOCARCINOGENESIS

PPs belong to a class of carcinogens whose mode of action does not involve direct damage to the DNA. Assays measuring covalent DNA binding of DNA adducts and short-term tests of mutagenicity have been uniformly negative when PPs were used (reviewed in 93). In addition, two of the most potent PPs, Wy-14,643 and ciprofibrate, were negative in classic initiation assays (94, 95). Therefore, to understand how PPs induce hepatic tumors in rodents, alternative mechanisms of action must be considered.

PPARα plays a central role in the hepatocarcinogenesis by PPs. Ligand binding studies with PPs of different affinities show a good correlation between PPARα binding or activation and its potency as a hepatocarcinogen (discussed above). An important step toward understanding the role of PPARα in PP-induced hepatocarcinogenesis came from the development of a mouse strain in which the PPARα gene was functionally inactivated by targeted disruption of the ligand-binding domain (96). Short-term treatment of PPARα-null mice with hypolipidemic PPs (96) or the phthalate ester plasticizers di-*n*-butyl phthalate and diethylhexylphthalate (31) failed to induce classical short-term responses associated with PP exposure, including peroxisome proliferation and transcriptional activation of peroxisomal β-oxidation and microsomal ω-oxidation genes. In this mouse model, in the liver, a large number of genes with diverse functions have also been shown to depend on a functional PPARα for constitutive and altered regulation by PPs (summarized in 97).

To determine if PPARα was necessary for PPs to induce liver cancer, wild-type and PPARα-null mice were fed for 11 months a diet containing 0.1% Wy-14,643. There was a 100% incidence of hepatic neoplasms in the treated wild-type mice (98). In contrast, none of the PPARα-null mice developed liver tumors. The mechanism through which PPARα mediates the carcinogenic effects remains to be elucidated. However, it is clear that this receptor plays a necessary role in both peroxisome proliferation and cell proliferation in the liver, both of which have been linked to the observed increases in hepatocellular tumors.

Two hypotheses have been proposed to account for PP-induced hepatocarcinogenesis in rodents (Figure 3). The oxidative stress hypothesis proposes that the carcinogenicity of PPs is initiated by oxidative damage due to excessive production of peroxisomal hydrogen peroxide (93). The alternative hypothesis centers around imbalances in hepatocyte growth control resulting from increases in cell proliferation and suppression of apoptosis (99, 100).

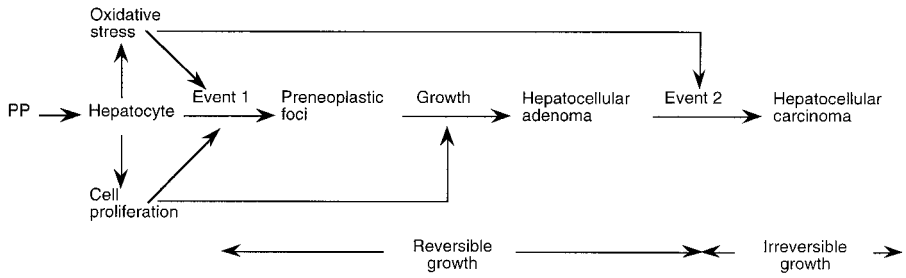

Figure 3 Cancer model for peroxisome proliferators (PPs). The different stages of PP-induced conversion of normal hepatocytes to hepatocellular carcinomas are shown. Two critical events (Event 1 and Event 2) are thought to occur that convert a normal hepatocyte to a hepatocellular carcinoma. Hepatocytes exposed to PPs have increases in oxidative stress and cell proliferation, which can enhance the probability of Event 1, converting a normal hepatocyte to an altered hepatocyte. Event 1 could arise through a mutagenic or an epigenetic process. In the presence of PPs, a subpopulation of altered cells exhibit increased cell proliferation and progress to become preneoplastic basophilic foci and, ultimately, hepatocellular adenomas. Cessation of exposure results in involution of these lesions through an increased rate of apoptosis. A mutational event (Event 2) likely drives conversion of adenomas to carcinomas because carcinomas persist after compound withdrawal.

The oxidative stress hypothesis is based on early observations that chronic exposure to PPs results in a sustained increase in oxidative stress in target rodent hepatocytes because of an imbalance between the production and degradation of hydrogen peroxide or other reactive oxygen species (101). PP treatment induces peroxisomal ACO and urate oxidase (93), and as a consequence of substrate oxidation, hydrogen peroxide is produced as a by-product. Under homeostatic conditions, hydrogen peroxide is neutralized by catalase; however, in this hypothesis, the excess hydrogen peroxide generated during PP exposure is not neutralized by a concomitant increase in catalase levels. The excess hydrogen peroxide may then diffuse through the peroxisomal membrane and attack DNA directly or via other reactive oxygen species (e.g. hydroxyl radical). 8-Hydroxydeoxyguanosine (8-OH-dG) is a frequently used marker of DNA damage produced by oxygen radicals. PPs increase 8-OH-dG levels in livers of treated rodents, but there is no clear link between the observed increase and carcinogenesis (102). The oxidative stress hypothesis predicts a central role for ACO in generating the PP-induced hydrogen peroxide. Mice that lack functional ACO exhibit PP-independent increases in the expression of genes normally regulated by PPARα (103). Furthermore, these mice have increased incidence of liver tumors (104). Based on these results, ACO has an insignificant role in PP-induced oxidative stress and was hypothesized to negatively regulate the levels of endogenous PPARα activators that have carcinogenic activity. Likely candidates include ω_6-unsaturated fatty acids and PGs that (*a*) activate PPARα, (*b*) are metabolized by the fatty acid

β-oxidation system, and (*c*) have growth-promoting effects (reviewed in 105). Therefore, although peroxisome proliferation and the accompanying increases in reactive oxygen species may play a role in the carcinogenicity of PPs, it is clear that oxidative damage alone is not sufficient for the hepatocarcinogenicity.

Carcinogenesis is a multistep process that can be conceptually divided into three stages: initiation, promotion, and progression (106). Initiation of a cell involves two steps: mutational events that irreversibly damage DNA, and fixation of these lesions through rounds of cell division. Initiated cells must undergo further genetic alterations before acquiring a malignant phenotype (106, 107). Cell proliferation induced by PPs has been extensively characterized and likely plays a central role in the hepatocarcinogenesis (Figure 3). PPARα is necessary for PP-mediated hepatocyte proliferation. Within 48 h of treatment, cell proliferation is increased in the livers of PP-treated rodents (108). Increases in cell proliferation in the liver of wild-type mice treated with Wy-14,643 in the diet for 1 or 5 weeks is abolished in PPARα-null mice (98). Although the mechanism of cell proliferation is unclear, recent work suggests that sustained PPARα-dependent alterations in cell cycle regulatory proteins play a role in PP-induced hepatocarcinogenesis (109). The early increase in cell replication induced by PPs could increase the frequency of spontaneous mutations (93), and these spontaneous mutations and those induced by exogenous sources could be fixed by further rounds of cell division or clonal expansion. With chronic Wy-14,643 treatment, cell proliferation in the liver is sustained; however, with weaker PPs [e.g. di(2-ethylhexyl)phthalate or nafenopin], cell proliferation in the liver returns to control levels (110, 111). In general, the magnitude of the sustained increase in cell proliferation during chronic PP treatment has been a good predictor of eventual tumor yield in rodents, but not when the level of exposure or potency of the PPs is low (100, 108, 110–112).

Elimination of initiated cells via apoptosis is one defense mechanism against neoplastic transformation. In addition to increasing hepatocellular proliferation, PPs decrease apoptosis in normal and initiated cell populations, and this likely contributes to the hepatocarcinogenic effects of PPs. Nafenopin and Wy-14,643 inhibit transforming growth factor-β1–induced apoptosis in vitro (113), and this suppressive effect of nafenopin could be ablated with increasing concentrations of a dominant negative PPARα (114). These data suggest that PPs may interfere with the mito-inhibitory and apoptotic effects of this cytokine through a PPARα-dependent mechanism (114).

PPs are commonly classified as tumor promoters. PPs can promote the clonal expansion of anchorage-independent hepatocytes in vitro (115, 116), which suggests that these compounds perturb the balance of mitosis and apoptosis, leading to the net outgrowth of initiated clones. PPs selectively stimulate growth of initiated cells exhibiting a phenotype different from cells composing either spontaneous tumors or tumors induced by other nongenotoxic chemicals (117). PP-induced foci are predominantly basophilic and do not express proteins such as glutathione S-transferase–placental form or γ-glutamyl transpeptidase, which

are normally associated with foci and tumors induced by other nongenotoxic carcinogens or DNA-damaging agents (118). Cell proliferation within PP-induced basophilic foci and adenomas is increased during PP exposure (94, 119, 120). Apoptosis is also increased in these foci and adenomas, but the lesions continue to grow because of an imbalance favoring cell replication over cell death (120a). Progression from initiated cell to hepatic carcinomas is dependent on the continued presence of the PPs. Five weeks after withdrawal of nafenopin there was a 20% reduction in the number of hepatocytes in the noninvolved tissue and 85% reduction of cells in foci, adenomas, and carcinomas (120). The data indicate that continual activation of PPARα is necessary for the growth and maintenance of initiated cells in foci, adenomas, and carcinomas in the liver of PP-treated rodents.

Although rodents are sensitive to the hepatocarcinogenic effects of PPs, there is little evidence that humans are at increased risk of liver cancer, even after chronic exposure. The hypolipidemic agents gemfibrozil and clofibrate have been clinically used for 15 and 30 years, respectively, and epidemiological studies do not reveal a statistically significant increase in cancer up to 8 years after initiation of therapy (93). Human liver contains a functionally active PPARα; however, the human PPARα is expressed to only about 10% of that in mouse liver, and extracts from human liver contain little PPARα that can bind to a PPRE (121). These dramatic differences in PPARα expression and activity may account for the absence of indicators of PP exposure in human liver, including increases in peroxisome proliferation and cell proliferation (93). Because the rodent hepatocarcinogenesis following PP exposure is mediated via PPARα, the current evidence suggests that humans exposed to these compounds are not at increased risk for developing liver tumors.

SUPPRESSION OF TUMOR GROWTH BY PEROXISOME PROLIFERATORS

Emerging evidence indicates that PPs have the ability to suppress the growth of different types of human cancer. Early indications that PPs can suppress growth of tumors came from traditional studies of the growth promotion effects of PPs in the livers of rats. Although PPs promote the growth of basophilic lesions (discussed above), there was a paradoxical suppression of both γ-glutamyl transpeptidase-positive and ATPase-deficient foci (122, 123), indicating that PPs through PPARs may inhibit growth in these lesions.

A large number of studies have demonstrated growth inhibition properties of PPARα and PPARγ ligands on human tumor cell lines. A large number of tumor types appear to be sensitive to PPs, including cells from prostate cancer (124), monocytic leukemia (125), ovarian carcinoma (126), hepatoma (127), liposarcoma (128), and breast cancer (129–131). Growth inhibition of these cell lines occurred through a number of distinct mechanisms, including increases in necro-

sis (124), apoptosis (127, 129), and growth arrest (125, 126). In addition, increases in differentiation to a cell type expressing markers of adipocyte phenotype also occurred in liposarcoma (128) and breast cancer (131) cell lines. Increases in PPAR expression do not seem to be required for growth inhibition, as PPARα was not altered in hepatoma cells (127) and PPARγ was unchanged in monocytic leukemia (125) and prostate cancer (137) cells. In all cases examined, however, PPARs were expressed to varying degrees in the target tissues (124, 127, 129, 131).

PPAR ligands have potent anticancer activity in vivo. In studies using immunodeficient mice injected with human prostate (124) or human breast (129) cancer cells, treatment of mice with the PPARγ ligand troglitazone decreased tumor volume and weight. It is interesting to note that treatment with a combination of troglitazone and all-*trans*-retinoic acid was more potent at inhibition of tumor growth in these two studies. Treatment with dehydroepiandrosterone, a primary steroid precursor and a PP, decreased the number of ethylnitrosourea-induced rat mammary tumors (130). Lastly, the intermediate-to-high-grade liposarcomas in patients treated with a thiazolidinedione exhibited extensive lipid accumulation and up-regulation of genes involved in terminal adipocyte differentiation, as well as down-regulation of a marker of cell proliferation (128). In summary, many different types of cancer may respond favorably to PP therapy, providing a rational basis for anticancer medicines that work through PPAR family members.

There is conflicting evidence of the effects of PP treatment on colon cancer cell growth. Similar to the therapeutic effects of PPs, discussed above, treatment of human colon cancer cell lines with troglitazone resulted in decreases in cell replication, in G1 cell-cycle arrest, and in increases in expression of markers of enterocyte cell differentiation (120a). Consistent with the therapeutic effects of PPs, mutations in PPARγ were found in human colon cancers that resulted in a decreased ability of PPARγ to be activated by ligands (133). These data indicate that a functional PPARγ is required for normal growth properties of human colon cells. In contrast, treatment of *Min* mice predisposed to intestinal neoplasia with the PPARγ ligands troglitazone or BRL-49,653 resulted in increases in the number of colon tumors but not small-intestine tumors (134, 135). Increases in the protein β-catenin, which has been linked to colon cancer, were observed in the colon in these mice after BRL-49,653 treatment, pointing to a reprogramming of gene expression important in tumorigenesis (135). Thus, the promise of using thiazolidinediones to treat colon cancer is overshadowed by the possibility that these compounds may actually increase susceptibility of colon cancer in certain human subpopulations. Further work on the significance of findings in the *Min* mice is needed.

PPARS AND INFLAMMATION

There is increasing evidence that PPARs are capable of inhibiting inflammatory responses in certain cell types. This effect may be mediated by at least two mechanisms. First, proinflammatory lipid metabolites may serve as ligands for PPARs,

thereby activating PPAR-responsive enzymes responsible for their clearance. This has been demonstrated for PPARα-mediated catabolism of LTB4 (13), and PPARγ-induced 12/15-lipoxygenase catabolism of linoleic acid and arachidonic acid (136) to PPARγ ligands. It is interesting to note that interleukin (IL)-4 up-regulates both 12/15-lipoxygenase and PPARγ, which suggests a new paradigm for the regulation of nuclear receptor function by cytokines. Second, PPARs may influence cytokine induction by other transcription factors with roles in mediating inflammation, such as signal tranducers and activators of transcription, NF-κB, and activator protein-1. This mechanism appears to be operative via PPARγ, as well as PPARα. For example, activation of PPARγ inhibits transcription of inducible nitric oxide synthase, gelatinase B, and scavenger receptor A in activated macrophages (137), and alpha tumor necrosis factor, IL-1β, and IL-6 in monocytes (138), by a mechanism that occurs in the absence of PPARγ-DNA interaction. In a similar fashion, activation of PPARα in activated aortic smooth muscle cells leads to decreased expression of IL-6 and cyclooxygenase 2 (COX-2) (14). Possible explanations for these effects include PPAR titration of essential transcriptional cofactors, such as CBP/p300 and SRC-1 (138, 14), used by other transcription factors, or perhaps by direct inactivation by PPAR-transcription factor interaction.

A direct role for PPARα in down-regulating inflammatory responses in vivo is now well established. Treatment of wild-type but not PPARα-null mice with diverse PPs resulted in down-regulation of a number of acute-phase response genes normally induced in the liver after a localized inflammatory stimulus (139, 140). In PPARα-null mice, acute-phase response genes are expressed at higher levels than in wild-type mice (139, 140), LTB4- and arachidonic acid-induced ear swelling is prolonged (13), and there are higher levels of age-associated NF-κB activity and regulated genes, including IL-6, IL-12, COX-2, and tumor necrosis factor alpha (TNFα) (141, 142). Similar responses may occur in humans because patients receiving therapeutic doses of hypolipidemic drugs have decreased acute-phase proteins and cytokine levels in serum (14). These data indicate that the PPAR family members may be attractive candidates for therapeutic intervention in chronic inflammatory diseases often associated with aging.

FUTURE DIRECTIONS

In the decade since the first PPAR was cloned, a substantial body of research has revealed that PPARs exist in three isoforms with distinct, sometimes overlapping, roles in regulating fatty acid metabolism, glucose homeostasis, cell growth and differentiation, and inflammation. Although much has been accomplished, many important questions remain. A large number of both endogenous and exogenous PPAR ligands have been identified. It is likely that additional natural ligands, and the conditions under which they are produced, will be uncovered. Considerable effort has already been directed toward understanding the complex interactions between PPARs, other nuclear receptors, and receptor cofactors. Forthcoming

research will address how the architecture of these complexes determines tissue-specific patterns of gene expression. This, in turn, should facilitate identification of additional genes that are both directly and indirectly regulated by the different PPARs. Clinically, PPs are already emerging as important mediators in the progression of certain chronic human diseases. Thus, it will be important to explore how these receptors can be manipulated therapeutically to delay disease progression or alleviate symptoms. Finally, given that several PPs are potent rodent carcinogens, another significant challenge will be to gain a more complete understanding of how accurately rodent cells predict human responses. If they are not good surrogates, it will be important to understand why. Answers to these questions will greatly enhance our ability to more accurately estimate the true relative risk of adverse health effects in humans receiving chronic therapeutic or environmental exposure to various PPs. Looking toward the future, research on the mechanisms of PPAR activation promises to continue to yield exciting and biomedically beneficial information.

ACKNOWLEDGMENTS

We thank Dr. Greg Kedderis for reviewing the manuscript, Kathy Claypoole for preparation of the manuscript, and Stan Piestrak for artwork. We apologize to our colleagues whose work we were not able to cite because of space limitations.

LITERATURE CITED

1. Lock EA, Mitchell AM, Elcombe CR. 1989. Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu. Rev. Pharmacol. Toxicol.* 29:145–63
2. Reddy JK, Azarnoff DL. 1980. Hypolipidemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283:397–98
3. Lapinskas PJ, Corton JC. 1998. Molecular mechanisms of hepatocarcinogenic peroxisome proliferators. In *Molecular Biology of the Toxic Response,* ed. A Puga, KB Wallace, pp. 219–53. Philadelphia, PA: Taylor & Francis
4. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leithersdort E, Fruchart JC. 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 98:2088–93
5. Kliewer SA, Willson TM. 1998. The nuclear receptor PPARgamma-bigger than fat. *Curr. Opin. Genet. Dev.* 5:576–81
6. Spiegelman BM, Flier, JS. 1996. Adipogenesis and obesity: rounding out the big picture. *Cell* 87:337–89
7. Spiegelman BM. 1998. PPARgamma in monocytes: less pain, any gain? *Cell* 93:153–55
8. Wolf G. 1999. The role of oxidized low-density lipoprotein in the activation of peroxisome proliferator-activated receptor gamma: implications for atherosclerosis. *Nutr. Rev.* 57:88–91
9. Reddy JK, Rao MS. 1986. Peroxisome proliferators and cancer: mechanisms and implications. *Trends Pharmacol. Sci.* 7:438–43
10. Issemann I, Green S. 1990. Activation of

a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 347:645–50
11. Lemberger T, Desvergne B, Wahli W. 1996. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu. Rev. Cell Dev. Biol.* 12:335–63
12. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, et al. 1995. The nuclear receptor superfamily: the second decade. *Cell* 83:835–39
13. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, et al. 1996. The PPARα-leukotriene B_4 pathway to inflammation control. *Nature* 384:39–43
14. Staels B, Wolfgang K, Habib A, Merval R, Lebret M, et al. 1998. Activation of human aortic smooth-muscle cells is inhibited by PPARα but not PPARγ activators. *Nature* 393:790–93
15. Lm H, Gupta RA, Ma W-g, Paria BC, Moller DE, et al. 1999. Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. *Genes Dev.* 13:561–74
16. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, et al. 1995. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR) gamma gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc. Natl. Acad. Sci. USA* 92:7921–25
17. MacDougald OA, Lane MD. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. *Annu. Rev. Biochem.* 64:345–73
18. Xin X, Yang S, Kowalski J, Gerritsen ME. 1999. Peroxisome proliferator-activated receptor γ ligands are potent inhibitors of angiogenesis in vitro and in vivo. *J. Biol. Chem.* 274:9116–21
19. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, et al. 1998. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* 395:137–43
20. Uppenberg J, Svensson C, Jaki M, Bertilsson G, Jendeberg L, et al. 1998. Crystal structure of the ligand binding domain of the human nuclear receptor PPAR-gamma. *J. Biol. Chem.* 273:31108–12
21. Xu HE, Lambert MH, Montana VG, Parks DJ, et al. 1999. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* 3:397–403
22. Moras D, Gronemeyer H. 1998. The nuclear receptor ligand-binding domain: structure and function. *Curr. Opin Cell Biol.* 3:384–91
23. Sorensen HN, Treuter E, Gustafsson JA. 1998. Regulation of peroxisome proliferator-activated receptors. *Vitam. Horm.* 54:121–66
24. Juge-Aubry CE, Hammar E, Siegrist-Kaiser C, Pernin A, Takeshita A, et al. 1999. Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor alpha by phosphorylation of a ligand-independent trans-activating domain. *J. Biol. Chem.* 274:10505–10
25. Hu E, Kim JB, Sarraf P, Spiegelman BM. 1996. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science* 274:2100–3
26. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, et al. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* 91:7355–559
27. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkson WO, Willson TM, et al. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). *J. Biol. Chem.* 270:12953–56
28. 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* 94:4312–17

29. Dowell P, Peterson VJ, Zabriskie TM, Leid M. 1997. Ligand-induced peroxisome proliferator-activated receptor α conformational change. *J. Biol. Chem.* 272:2013–20
30. Kliewer SA, Lenhard JM, Wilson TM, Patel I, Morris DC, et al. 1995. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* 83:813–19
31. Lapinskas PJ, Corton JC. 1997. Phthalate ester plasticizers differentially activate the peroxisome proliferator-activated receptors. *Fundam. Appl. Toxicol.* 36:144
32. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, et al. 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* 11:779–91
33. Kliewer SA, Lenhard JM, Wilson TM, Patel I, Morris DC, et al. 1995. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* 83:813–19
34. Elbrecht A, Chen Y, Adams A, Berger J, Griffin P, et al. 1999. L-764406 is a partial agonist of human peroxisome proliferator-activated receptor gamma. The role of Cys313 in ligand binding. *J. Biol. Chem.* 274:7913–22
35. Oberfield JL, Collins JL, Holmes CP, Goreham DM, Cooper JP, et al. 1999. A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation. *Proc. Natl. Acad. Sci. USA* 96:6102–6
36. Berger J, Leibowitz MD, Doebber TW, Elbrecht A, Zhang B, et al. 1999. Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPAR delta ligands produce distinct biological effects. *J. Biol. Chem.* 274:6718–25
37. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. 1997. Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.* 272:3406–10
38. Gottlicher M, Widmark E, Li Q, Gustafsson J-Å. 1992. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* 89:4653–57
39. Krey G, Keller H, Mahfoudi A, Medin J, Ozato K, et al. 1993. Xenopus peroxisome proliferator activated receptors: genomic organization, response element recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *J. Steroid Biochem.* 47:65–73
40. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, et al. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. USA* 94:4318–23
41. Yu K, Bayona W, Kallen CB, Hardings HP, Ravera CP, et al. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* 270:23975–83
42. Karmali RA. 1986. Eicosanoids and cancer. *Prog. Clin. Biol. Res.* 222:687–97
43. Diczfalusy UG, Alexson SEH. 1992. Role of peroxisomes in the degradation of prostaglandins. In *New Developments in Fatty Acid Oxidation*, ed. PM Coates, K Tanaka, pp. 253–61. New York: Wiley-Liss
44. Leug LK, Glauert HP. 1996. Reduction of the concentrations of prostaglandins E2 and F2α, and thromboxane B2 in cultured rat treated with the peroxisome proliferator ciprofibrate. *Toxicol. Lett.* 85:143–49
45. Nagy L, Tontonoz P, Alvarex JG, Chen H, Evans RM. 1998. Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR-gamma. *Cell* 93:229–40

46. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 93:241–52
47. Tugwood JD, Aldridge TC, Lambe KG, Macdonald N, Woodyatt NJ. 1996. Peroxisome proliferator-activated receptors: structures and function. *Ann. NY Acad. Sci.* 27:252–65
48. Osumi T, Osada S, Tsukamoto T. 1996. Analysis of peroxisome proliferator-responsive enhancer of the rat acyl-CoA oxidase gene. *Ann. NY Acad. Sci.* 804:202–13
49. Gervois P, Chopin-Delannoy S, Fadel A, Dubois G, Kosykh V, et al. 1999. Fibrates increase human REV-ERB alpha expression in liver via a novel peroxisome proliferator-activated receptor response element. *Mol. Endocrinol.* 3:400–9
50. Nunez SB, Medin JA, Braissant O, Kemp L, Wahli W, et al. 1997. Retinoid X receptor and peroxisome proliferator-activated receptor activate an estrogen responsive gene independent of the estrogen receptor. *Mol. Cell. Endocrinol.* 127:27–40
51. Palmer CAN, Hsu M-H, Griffin KJ, Johnson EF. 1995. Novel sequence determinants in peroxisome proliferator signaling. *J. Biol. Chem.* 270:16114–21
52. Varanasi U, Chu R, Huang Q, Castellon R, Yeldandi AV, et al. 1996. Identification of a peroxisome proliferator-responsive element upstream of the human peroxisomal fatty acyl coenzyme A oxidase gene. *J. Biol. Chem.* 271:2147–55
53. Johnson EF, Palmer CNA, Griffin KJ, Hsu M-H. 1996. Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. *FASEB J.* 10:1241–48
54. Kliewer SA, Umeson K, Noonan DJ, Heyman RA, Evans RM. 1992. Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways through heterodimer of formation of their receptors. *Nature* 358:771–74
55. Gearing KL, Gottlicher M, Teboul M, Widmark E, Gustafsson J-Å. 1993. Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor. *Proc. Natl. Acad. Sci. USA* 90:1440–44
56. Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, et al. 1993. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA* 90:2160–64
57. Mukherjee R, Davies PJ, Crombie DL, Bischoff ED, Cesario RM, et al. 1997. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* 386:407–10
58. Canan Koch SS, Dardashti LJ, Cesario RM, Croston GE, Boehm MF, et al. 1999. Synthesis of retinoid X receptor-specific ligands that are potent inducers of adipogenesis in 3T3-L1 cells. *J. Med. Chem.* 42:742–50
59. Standeven AM, Escobar M, Beard RL, Yuan YD, Chandraratna RA. 1997. Mitogenic effect of retinoid X receptor agonists in rat liver. *Biochem. Pharmacol.* 54:517–24
60. Juge-Aubry CE, Gorla-Bajszczak A, Pernin A, Lemberger T, Wahli W, et al. 1995. Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. *J. Biol. Chem.* 270:18117–22
61. Chu R, Madison LD, Lin Y, Kopp P, Rao MS, et al. 1995. Thyroid hormone (T_3) inhibits ciprofibrate-induced transcription of genes encoding β-oxidation enzymes: cross talk between peroxisome proliferator and T_3 signaling pathways. *Proc. Natl. Acad. Sci. USA* 92:11593–97
62. Jow L, Mukherjee R. 1995. The human peroxisome proliferator-activated recep-

tor (PPAR) subtype NUC1 represses the activation of hPPAR alpha and thyroid hormone receptors. *J. Biol. Chem.* 270:3836–40
63. Hunter J, Kassam A, Winrow CJ, Rachubinski RA, Capone JP. 1996. Crosstalk between the thyroid hormone and peroxisome proliferator-activated receptors in regulating proliferator-response genes. *Mol. Cell. Endocrinol.* 116:213–21
64. Masuda N, Yasumo H, Tamura T, Hashiguchi N, Furusawa T, et al. 1997. An orphan nuclear receptor lacking a zinc-finger DNA-binding domain: interaction with several nuclear receptor. *Biochim. Biophys. Acta* 1350:27–32
65. Miyata KS, McCaw SE, Patel HV, Rachubinski RA, Capone JP. 1996. The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J. Biol. Chem.* 271:9189–92
66. Chu R, Lin Y, Rao MS, Reddy JK. 1996. Cloning and identification of rat deoxyuridine triphosphatase as an inhibitor of peroxisome proliferator-activated receptor α. *J. Biol. Chem.* 271:27670–76
67. Sakai M, Matsushima-Hibiya Y, Nishizawa M, Nishi S. 1995. Suppression of rat glutathione transferase P expression by peroxisome proliferators: interaction between jun and peroxisome proliferator-activated receptor alpha. *Cancer Res.* 55:5370–76
68. Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, Leid M. 1997. p300 functions as a coactivator for the peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.* 272:33435–43
69. Henry KW, Yuan X, Koszewski NJ, Onda H, Kwiatkowski DJ, et al. 1998. Tuberous sclerosis gene 2 product modulates transcription mediated by steroid hormone receptor family members. *J. Biol. Chem.* 273:20535–39
70. Zhu Y, Qi C, Jain S, Sambasiv RM, Reddy JK. 1997. Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. *J. Biol. Chem.* 272:25500–6
71. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92:829–39
72. Castillo G, Brun RP, Rosenfield JK, Hauser S, Park CW, et al. 1999. An adipogenic cofactor bound by the differentiation domain of PPARγ. *EMBO J.* 13:3676–87
73. Heinlein CA, Ting HJ, Yeh S, Chang C. 1999. Identification of ARA70 as a ligand-enhanced coactivator for the peroxisome proliferator-activated receptor γ. *J. Biol. Chem.* 23:16147–52
74. Zhu Y, Qi C, Calandra C, Rao MS, Reddy JK. 1996. Cloning and identification of mouse steroid receptor coactivator-1 (MSRC-1), as a coactivator of peroxisome proliferator-activated receptor gamma. *Gene Exp.* 6:185–95
75. DiRenzo J, Söderström M, Kurokawa R, Ogliastro M-H, Ricote M, et al. 1997. Peroxisome proliferator-activated receptors and retinoic acid receptors differentially control the interactions of retinoid X receptor heterodimers with ligands, coactivators, and corepressors. *Mol. Cell. Biol.* 17:2166–76
76. Qi C, Zhu Y, Pan J, Yeldandi AV, Rao MS, et al. 1999. Mouse steroid receptor coactivator-1 is not essential for peroxisome proliferator-activated receptor alpha-regulated gene expression. *Proc. Natl. Acad. Sci. USA* 96:1585–90
77. Meertens LM, Miyata KS, Cechetto JD, Rachubinski RA, Capone JP. 1998. A mitochondrial ketogenic enzyme regulates its gene expression by association with the nuclear hormone receptor PPARα. *EMBO J.* 23:6972–78
78. Sande S, Privalsky M. 1996. Identification of TRACs (T_3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity

of, nuclear hormone receptors. *Mol. Endocrinol.* 10:813–25
79. Seol W, Mahon MJ, Lee Y-K, Moore DD. 1996. Two receptor interacting domains in the nuclear hormone receptor corepressor RIP13/N-CoR. *Mol. Endocrinol.* 10:1646–55
80. Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, et al. 1999. Identification of nuclear receptor corepressor as a peroxisome proliferator-activated receptor alpha interacting protein. *J. Biol. Chem.* 274:15901–7
81. Zamir I, Zhang J, Lazar MA. 1997. Stoichiometric and steric principles governing repression by nuclear hormone receptors. *Genes Dev.* 11:835–46
82. Treuter E, Albrektsen T, Johansson L, Leers J, Gustafsson JA. 1998. A regulatory role for RIP140 in nuclear receptor activation. *Mol. Endocrinol.* 12:864–81
83. Miyata KS, McCaw SE, Meertens LM, Patel HV, Rachubinski RA, et al. 1998. Receptor-interacting protein 140 interacts with, and inhibits transactivation by, peroxisome proliferator-activated receptor alpha and liver-X-receptor alpha. *Mol. Cell. Endocrinol.* 146:69–76
84. Jansen JH, Mahfoudi A, Rambaud S, Lavau C, Wahli W, Dejean A. 1995. Multimeric complexes of the PML-retinoic acid receptor α fusion protein in acute promyelocytic leukemia cells and interference with retinoid and peroxisome-proliferator signaling pathways. *Proc. Natl. Acad. Sci. USA* 92:7401–5
85. Miyata KS, Zhang B, Marcus SL, Capone JP, Rachubinski RA. 1993. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds to a peroxisome proliferator-responsive element and antagonizes peroxisome proliferator-mediating signaling. *J. Biol. Chem.* 268:19169–72
86. Marcus SL, Capone JP, Rachubinski RA. 1996. Identification of COUP-TFII as a peroxisome proliferator response element binding factor using genetic selection in yeast: COUP-TFII activates transcription in yeast but antagonizes PPAR signaling in mammalian cells. *Mol. Cell. Endocrinol.* 120:31–39
87. Miyamoto T, Kaneko A, Kakizawa T, Yajima H, Kamijo K, et al. 1997. Inhibition of peroxisome proliferator signaling pathways by thyroid hormone receptor. *J. Biol. Chem.* 272:7752–58
88. Yan ZH, Karam WG, Staudinger JL, Medvedev A, Ghanayem BI, et al. 1999. Regulation of peroxisome proliferator-activated receptor alpha-induced transactivation by the nuclear orphan receptor TAK1/TR4. *J. Biol. Chem.* 273:10948–57
89. Hertz R, Bishara-Shieban J, Bar-Tana J. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs: suppression of apolipoprotein C-III. *J. Biol. Chem.* 270:13470–75
90. Hertz R, Seckback M, Zaki MM, Bar-Tana J. 1996. Transcriptional suppression of the transferrin gene by hypolipidemic peroxisome proliferators. *J. Biol. Chem.* 271:218–24
91. Vu-Dac N, Chopin-Delannoy S, Gervois P, Bonnelye E, Martin G, et al. 1998. The nuclear receptors peroxisome proliferator-activated receptor α and rev-erbα mediate the species-specific regulation of apolipoprotein A-1 expression by fibrates. *J. Biol. Chem.* 273:25713–20
92. Shyamala G, Schneider W, Schott D. 1990. Developmental regulation of murine mammary progesterone receptor expression. *Endocrinology* 126:2882–89
93. Ashby J, Brady A, Elcombe CR, Elliott BM, Ishnmael J, et al. 1994. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* 13:S1–117
94. Cattley RC, Marsman DS, Popp JA. 1989. Failure of the peroxisome proliferator WY-14,643 to initiate growth-selectable foci in rat liver. *Toxicology* 56:1–7
95. Glauert HP, Clark TD. 1989. Lack of ini-

tiating activity of the peroxisome proliferator clofibrate in two-stage hepatocarcinogenesis. *Cancer Lett.* 44:95–100
96. Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, et al. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferators. *Mol. Cell. Biol.* 15:3012–22
97. Gonzalez FJ, Peters JM, Cattley RC. 1998. Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activated receptor α. *J. Natl. Cancer Inst.* 90:1702–9
98. Peters JM, Cattley RC, Gonzalez FJ. 1997. Role of PPARα in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* 18:2029–33
99. Bursch W, Lauer LB, Timmermann-Troseiner, Barthel G, Schuppler J, et al. 1984. Controlled death (apoptosis) of normal and putative preneoplastic cells in rat liver following withdrawal of tumor promoters. *Carcinogenesis* 5:453–58
100. Marsman DS, Goldsworthy TL, Popp JA. 1992. Contrasting hepatocyte peroxisome proliferation, lipofuscin accumulation and cell turnover for the hepatocarcinogens Wy-14,643 and clofibric acid. *Carcinogenesis* 13:1011–17
101. Reddy JK, Rao MS. 1986. Peroxisome proliferators and cancer: mechanisms and implications. *Trends. Pharmacol. Sci.* 7:438–43
102. Sausen PJ, Lee DC, Rose ML, Cattley RC. 1995. Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: relationship to mitochondrial alterations. *Carcinogenesis* 16:1795–801
103. Fan C-Y, Pan J, Chu R, Lee D, Kluckman KD, et al. 1996. Hepatocellular and hepatic peroxisome alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene. *J. Biol. Chem.* 271:24698–710
104. Fan C-Y, Pan J, Usuda N, Yeldandi AV, Rao MS, et al. 1998. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase: implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. *J. Biol. Chem.* 273:15639–45
105. Masters C. 1996. Omega-3 fatty acids and the peroxisome. *Mol. Cell. Biochem.* 165:83–93
106. Farber E, Sarma DSR. 1987. Hepatocarcinogenesis: a dynamic cellular perspective. *Lab. Invest.* 56:4–22
107. Barrett JC. 1993. Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Env. Health Perspect.* 100:9–20
108. Marsman DS, Cattley RC, Conway JG, Popp JA. 1988. Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643) in rats. *Cancer Res.* 48:6739–44
109. Peters JM, Aoyama T, Cattley RC, Nobumitsu U, Hashimoto T, et al. 1998. Role of peroxisome proliferator-activated receptor α in altered cell cycle regulation in mouse liver. *Carcinogenesis* 19:1989–94
110. Eacho PI, Lanier TL, Brodhecker CA. 1991. Hepatocellular DNA synthesis in rats given peroxisome proliferating agents: comparison of Wy-14,643 to clofibric acid, nafenopin and LY171883. *Carcinogenesis* 12:1557–61
111. Barrass NC, Price RJ, Lake BG, Orton TC. 1993. Comparison of the acute and mitogenic effects of the peroxisome proliferator methylclofenapate and clofibric acid in rat liver. *Carcinogenesis* 14:1451–56
112. Tanaka K, Smith PF, Stromberg PC, Eydelloth RS, Herold EG, et al. 1992.

Studies of early hepatocellular proliferation and peroxisomal proliferation in Sprague-Dawley rats treated with tumorigenic doses of clofibrate. *Toxicol. Appl. Pharmacol.* 116:71–77

113. Bayly AC, Roberts RA, Dive C. 1994. Suppression of liver cell apoptosis in vitro by the non-genotoxic hepatocarcinogen and peroxisome proliferator nafenopin. *J. Cell Biol.* 125:197–203
114. Roberts RA, James NH, Woodyatt NJ, Nacdonald N, Tugwood JD. 1998. Evidence for the suppression of apoptosis by the peroxisome proliferator activated receptor alpha (PPARα). *Carcinogenesis* 19:43–48
115. James NH, Roberts RA. 1994. The peroxisome proliferator class of non-genotoxic hepatocarcinogens synergize with epidermal growth factor to promote clonal expansion of initiated rat hepatocytes. *Carcinogenesis* 15:2687–94
116. James NH, Ashby S, Roberts RA. 1995. Enhanced hepatocyte colony growth in soft agar after in vivo treatment with a genotoxic carcinogen: a potential assay for hepatocarcinogens? *Cancer Lett.* 93:121–28
117. Rao MS, Tatematsu M, Subbarao V, Ito N, Reddy JK. 1986. Analysis of peroxisome proliferator-induced preneoplastic and neoplastic lesions of rat liver for placental form of glutathione S-transferase γ-glutamyltranspeptidase. *Cancer Res.* 46:5287–90
118. Rao MS, Nenali MR, Usuda N, Scarpelli DG, Makino T, et al. 1988. Lack of expression of glutathione-s-transferase p, gamma-glutamyl transpeptidase and alpha-fetoprotein messenger RNAs in liver tumors induced by peroxisome proliferators. *Cancer* 48:4919–25
119. Grasl-Kraupp B, Huber W, Just W, Gibson G, Schulte-Hermann R. 1993. Enhancement of peroxisomal enzymes, cytochrome P-450 and DNA synthesis in putative preneoplastic foci of rat liver treated with the peroxisome proliferator nafenopin. *Carcinogenesis* 14:1007–12
120. Grasl-Kraupp B, Ruttkay-Nedecky B, Mullauer L, Taper H, Huber W, et al. 1997. Inherent increase of apoptosis in liver tumors: implications for carcinogenesis and tumor regression. *Hepatology* 25:906–12
120. (a) Kitamura S, Miyazaki Y, Shinomura Y, Kondo S, Kanayama S, et al. 1999. Peroxisome proliferator-activated receptor gamma induces growth arrest and differentiation markers of human colon cancer cells. *Jpn. J. Cancer Res.* 90:75–85
121. Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF. 1998. Peroxisome proliferator activated receptor-alpha expression in human liver. *Mol. Pharmacol.* 53:14–22
122. DeAngelo AB, Queral AE, Garrett CT. 1985. Concentration-dependent inhibition of development of GGT positive foci in rat liver by the environmental contaminant di(2-ethylhexyl) phthalate. *Environ. Health Perspect.* 60:381–85
123. Cattley RC, Popp JA. 1989. Differences between the promoting activities of the peroxisome proliferator WY-14,643 and phenobarbital in rat liver. *Cancer Res.* 49:3246–51
124. Kubota T, Koshizuka K, Williamson EA, Hiroya A, Said JW, et al. 1998. Ligand for peroxisome proliferator-activated receptor γ (troglitazone) has potent antitumor effect against human prostate cancer both *in vitro* and *in vivo*. *Cancer Res.* 58:3344–52
125. Zhu L, Gong B, Bisgaier CL, Aviram M, Newton RS. 1998. Induction of PPAR-gamma1 expression in human THP-1 monocytic leukemia cells by 9-cis-retinoic acid is associated with cellular growth suppression. *Biochem. Biophys. Res. Commun.* 251:842–48
126. Ferrandina G, Melichar B, Loercher A, Verschraegen CF, Kudelka AP, et al. 1997. Growth inhibitory effects of

sodium phenylacetate (NSC 3039) on ovarian carcinoma cells *in vitro*. *Cancer Res.* 57:4309–15
127. Canuto RA, Muzio G, Bonelli G, Maggiora M, Autelli R, et al. 1998. Peroxisome proliferators induce apoptosis in hepatoma cells. *Cancer Detect. Prev.* 22:357–66
128. Demetri GD, Fletcher CDM, Mueller E, Sarraf P, Naujoks R, et al. 1999. Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-γ ligand troglitazone in patients with liposarcoma. *Proc. Natl. Acad. Sci. USA* 96:3951–56
129. Elstner E, Muller C, Koshizuka K, Williamson EA, Park D, et al. 1998. Ligands for peroxisome proliferator-activated receptor γ and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cell *in vitro* and in BNX mice. *Proc. Natl. Acad. Sci. USA* 95:8806–11
130. Lubet RA, Gordon GB, Prough RA, Lei XD, You M, et al. 1998. Modulation of methylnitrosourea-induced breast cancer in Sprague Dawley rats by dehydroepiandrosterone: dose-dependent inhibition, effects of limited exposure, effects on peroxisomal enzymes, and lack of effects on levels of Ha-Ras mutations. *Cancer Res.* 58:921–26
131. Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, et al. 1998. Terminal differentiation of human breast cancer through PPAR gamma. *Mol. Cell* 1:465–70
132. Deleted in proof
133. Sarraf P, Mueller E, Smith WM, Wright HM, Kum JB, et al. 1999. Loss-of-function mutations in PPARγ associated with human colon cancer. *Mol. Cell* 3:799–804
134. Saez E, Tontonoz P, Nelson MC, Alvarez JGA, U TM, et al. 1998. Activators of the nuclear receptor PPARγ enhance colon polyp formation. *Nat. Med.* 4:1058–61
135. Lefebvre AM, Chen I, Desreumaux P, Najib J, Fruchart JC, et al. 1998. Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APC^{min}/+ mice. *Nat. Med.* 4:1053–57
136. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, et al. 1999. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/156-lipoxygenase. *Nature* 400:378–82
137. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. 1998. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 391:79–82
138. Jiang C, Ting AT, Seed B. 1998. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391:82–86
139. Corton JC, Fan LQ, Brown S, Anderson SP, Bocos C, et al. 1998. Down-regulation of cytochrome P450 2C family members and positive acute-phase response gene expression by peroxisome proliferator chemicals. *Mol. Pharmacol.* 54:463–73
140. Anderson SP, Cattley RC, Corton JC. 1999. Upregulation of acute-phase protein gene expression in peroxisome proliferator-induced hepatocellular adenomas in the rat. *Mol. Carcinog.* 26: 226–38
141. Spencer NF, Poynter ME, Im SY, Daynes RA. 1997. Constitutive activation of NF-kappa B in an animal model of aging. *Int. Immunol.* 9:1581–88
142. Poynter ME, Daynes RA. 1998. Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappa B signaling, and reduces inflammatory cytokine production in aging. *J. Biol. Chem.* 273:32833–41