0163-769X/99/$03.00/0
Endocrine Reviews 20(5): 649–688
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Peroxisome Proliferator-Activated Receptors: Nuclear Control of Metabolism*

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I. Introduction

THE REGULATION of lipid and carbohydrate metabolism is central to energy homeostasis in higher multicellular organisms. It involves control systems that are sensitive to stimuli such as the availability of food, physical activity, stress, light, and temperature. The coordination of the responses to signals triggered by these stimuli must occur on several levels to ensure a well adapted energy balance, ranging from hypothalamic functions in the brain to the direct control by lipids and carbohydrates of their own fate. Another important role for lipids is the ability of some of their metabolites, such as leukotrienes or prostaglandins, to be secreted and act as potent mediators in many biological processes that participate in the diverse responses to endogenous and exogenous challenges that the organism faces. In this article, we will concentrate on the role of lipids and their derivatives in the genetic control of their own systemic transport, cellular uptake, storage, mobilization, and use. Fine tuning of these metabolic processes is a hallmark of healthy organisms.

Lipid homeostasis depends on factors that are able to transduce metabolic parameters into regulatory events representing the fundamental components of the general control system. Such factors may modulate the catalytic activity of individual enzymes by allosteric interactions, as do citrate and palmitoyl-coenzyme A (CoA), which activate and inhibit the lipogenic enzyme acetyl-CoA carboxylase, respectively. Alternatively, these factors may participate directly in the transcriptional control of genes encoding proteins involved in key metabolic steps. Several transcription factors that sense lipid levels in animal cells have received much attention in recent years. The adipocyte determination and differentiation factor/sterol regulatory element-binding proteins (ADD/SREBPs)[1] are intracellular membrane-bound transcription factors whose activity is regulated by the cellular sterol content. In situations of sterol depletion, the active portion of SREBPs is released by proteolytic cleavage, enters the cell nucleus, and stimulates transcription of genes participating in three pathways of lipid metabolism: choles-

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* The work done in the authors' laboratories was supported by grants from the Swiss National Science Foundation and the Etat de Vaud.

[1] Abbreviations used: ACO, acyl-CoA oxidase; ACS, acyl-CoA synthase; ADD1, adipocyte determination and differentiation factor 1; AF1, AF2, activation function 1 and 2; BMI, body mass index; CAP, c-Cbl-associated protein; CARLA, coactivator-dependent receptor ligand assay; CBP, CREB-binding protein; C/EBP, CAAT/enhancer binding protein; CPT, carnitine palmitoyl transferase; CTE, carboxy terminal extension; COX, cyclo-oxygenase; DBD, DNA binding domain; DPSA, differential protease sensitivity assay; DR1 and DR2, direct repeat with 1 bp or 2 bp spacing, respectively; DRIP, vitamin D receptor interacting protein; DTA, diphtheria toxin A; ER, estrogen receptor; ERE, estrogen response element; FA, fatty acid; FABP, fatty acid binding protein; FAS, fatty acid synthase; FAT, fatty acid translocase; FATP, fatty acid transporter protein; FXR, farnesol X-activated receptor; GR, glucocorticoid receptor; H1 to H12, helices 1 to 12 (in nuclear receptor LBD); HAF, helix comprising the activation function domain; HETE, hydroxyeicosatetraenoic acid; HNF4, hepatocyte nuclear factor 4; HDL, high-density lipoprotein; KO, knock-out; LBD, ligand binding domain; LDL, low density lipoprotein; LIC, ligand induced complex; LPL, lipoprotein lipase; LTB4, leukotriene B4; LXRs: liver X receptors; MAP, microtubule-associated protein; MCAD, medium-chain acyl CoA dehydrogenase; mHMG-CoAS, mitochondrial hydroxymethylglutaryl-CoA; N-CoR, nuclear receptor corepressor; NSAID, nonsteroidal antiinflammatory drug; PBP, PPAR-binding protein; PEPCK, phosphoenolpyruvate carboxykinase; PDGF, platelet-derived growth factor; PGC1: PPARγ coactivator 1; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PUFA, polyunsaturated fatty acid; RA, retinoic acid; RAR, retinoic acid receptor; RIP140, receptor interacting protein 140; RXR, retinoid X receptor; SMRT, silencing mediator for retinoid and thyroid hormone receptors; SRC-1, steroid receptor coactivator 1; SREBPs, sterol regulatory element-binding proteins; TLS-CHOP, translocation liposarcoma-C/EBP homologous protein; TNF, tumor necrosis factor; TR, thyroid hormone receptor; TRAP, thyroid hormone receptor associated protein; TZD, thiazolidinedione; UCP, uncoupling protein; VDR, vitamin D receptor; VLDL, very low-density lipoprotein; ZDF, Zucker diabetic fatty.

terol biosynthesis, uptake of circulating fatty acids and cholesterol, and fatty acid biosynthesis (1). Another class of transcription factors comprises the liver X receptors [LXRs, *i.e.*, NR1H3, according to the unified nomenclature of nuclear hormone receptors (2)], whose ligands are oxidized derivatives of cholesterol (oxysterols). Analysis of LXRα-deficient mice revealed an essential function of this receptor as a major sensor of dietary cholesterol in the liver and an indispensable regulator of cholesterol homeostasis (3). Finally, the peroxisome proliferator-activated receptors (PPARs; NR1C) on which this review is focused belong to the steroid/thyroid/retinoid receptor superfamily, like LXRs, and are nuclear lipid-activable receptors that control a variety of genes in several pathways of lipid metabolism, including fatty acid transport, uptake by the cells, intracellular binding and activation, as well as catabolism (β-oxidation and ω-oxidation) or storage. In addition to being indeed activated by fatty acids, they respond to fibrate hypolipidemic drugs and to insulin sensitizers. Rapid progress has been made in the exploration of PPAR biology, which indicates new mechanisms for the regulation of lipid metabolism and functions. In this review, we will first describe molecular aspects concerning the genes that encode PPARs, their structure, and their mechanism of action. The second part concentrates on physiological aspects related to PPAR expression, target genes, and functional consequences of their activation, leading the discussion to the most recent developments in the understanding of their possible physiological roles.

II. Molecular Aspects

A. PPAR isotypes: identity, genomic organization, and chromosomal localization

Three related PPAR isotypes have been identified in vertebrates, including *Xenopus*, mouse, rat, hamster, and human (4–15). They were named PPARα (NR1C1), PPARβ (NR1C2), and PPARγ (NR1C3) when the group of three was originally found in *Xenopus* (15), shortly after the characterization of a first PPAR in the mouse (5). With respect to this isotype nomenclature established with the *Xenopus* PPARs, the mammalian PPARα and PPARγ were easily identified, while the third isotype was less clearly homologous to PPARβ and was alternatively called PPARδ, FAAR, or NUCI. Some evidence such as the expression pattern and the ligand pharmacological profile argue for these *Xenopus* and mammalian isotypes as being homologs. The analysis of the chicken PPAR β-like isotype also suggests that β and δ are indeed homologous since the chicken sequence falls about half way, in terms of similarity, between that of *Xenopus* and mammals (K. Umesono, personal communication). Below, we refer to this third isotype as PPARβ until additional data provide a final answer to this still open question of isotype identity.

Phylogenetic studies have shown that PPARs form a subfamily of the nuclear receptor superfamily, along with the receptors for thyroid hormone, retinoic acid (RA), vitamin D, ecdysone, and the orphan receptors Rev-ErbAα (=ear1; NR1D1) and E75 (NR1D3, from *Drosophila*), the two latter being the closest relatives of the PPARs (16). The ancestral genes in this subfamily appeared more than 500 million years ago (17), and a more recent second period saw the duplication of the ancestral thyroid hormone receptor (TR) gene into two genes, TRα (NR1A1) and TRβ (NR1A2), and of the ancestral retinoic acid receptor (RAR) gene into three genes, RARα (NR1B1), RARβ (NR1B2), and RARγ (NR1B3). Similarly, the three PPAR loci, α, β, and γ, appeared during this second period (16). Although it is not known whether the duplication events that produced the isotypes occurred exactly at the same time for the three receptors, they were likely contemporaneous to the appearance of the early vertebrates (18), as is indeed suggested by the chromosomal location of the TR, PPAR, and RAR genes. Since homologous isotypes have been found both in *Xenopus* and mammals for each of the three groups of genes, PPAR, RAR, and TR, the *Xenopus*-mammalian lineage divergence event can be used as a starting time point to determine the speed of evolution up to the present time within each of the three groups. In light of the amino acid sequence differences between the *Xenopus* and mammalian homologs, it appears that the PPAR genes have evolved 2–3 times faster than the RAR and TR genes. The possible relationship between this relatively rapid evolution and some particularities of PPAR ligand-binding properties will be discussed later.

The chromosomal localization of the PPAR genes has been defined in human and mouse. The human (h) PPARα was mapped on chromosome 22 slightly telomeric to a linkage group of six genes and genetic markers that are located in the general region 22q12-q13.1 (13). The hPPARγ gene is located on chromosome 3 at position 3p25, close to RARβ and TRβ, which are at positions 3p24 and 3p21, respectively (14). Furthermore, the gene is within 1.5 megabases (Mb) of D3S1263, which is a suitable polymorphic marker that could be used for linkage analysis to evaluate a potential contribution of PPARγ to lipid metabolism-related diseases (19). Finally, the hPPARβ has been assigned to chromosome 6, at position 6p21.1-p21.2 (20). In the mouse, PPARγ is located on chromosome 6 at position E3-F1, while PPARα and PPARβ are found on chromosome 15 and 17, respectively (21).

The mouse and hPPAR genes characterized to date reveal a common organization of the translated region in six coding exons with the following distribution: one exon for the N-terminal A/B domain, two exons for the DNA-binding domain (DBD)– one for each of the two zinc fingers–, one exon for the hinge region, and two exons for the ligand-binding domain (LBD). The mouse PPARα gene spans at least 30 kb and comprises a total of eight exons, with two exons corresponding to the 5′-untranslated region and the last exon of the LBD comprising the 3′-untranslated region (22). For the PPARβ gene, only a partial organization in *Xenopus*, which corresponds to the six exons of the translated region, has been reported so far (23). The human and mouse PPARγ genes extend over more than 100 kb of genomic DNA and give rise to three mRNAs, PPARγ1, PPARγ2, and PPARγ3, that differ at their 5′-end as a consequence of alternate promoter usage and splicing (Fig. 1). PPARγ1 is encoded by eight exons, comprising two γ1-specific exons for the 5′-untranslated region, A1 and A2, and the six coding exons that are common to all three mRNAs. PPARγ2 is encoded in seven exons, the first one, exon B, comprising the γ2 5′-untranslated region and encoding additional N-terminal

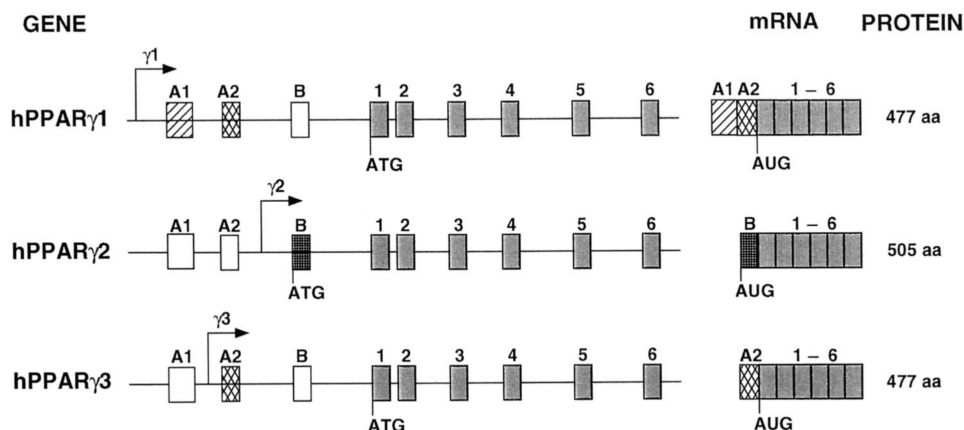

FIG. 1. hPPARγ isoforms. The genomic organization of the hPPARγ gene is shown. Two PPARγ isoforms are produced by the differential use of three promoters and alternative splicing of the three 5′-exons A1, A2, and B1. Exons 1–6 are common to all three transcripts. A scheme of the three mRNAs obtained is drawn, and the size of the proteins obtained is indicated. Note that transcription from the promoters γ1 and γ3 results in the same protein of 477 amino acids. In the human gene, the splicing event between exon A2 and exon 1 generates an AUG translation initiation codon six nucleotides upstream of the one used in other species, which is located at the very beginning of exon 1. Therefore, the hPPARγ1 protein has two additional amino acids at its N terminus compared with the rodent PPARγ1 protein. The PPARγ2 protein of 505 amino acids is produced by transcription from the promoter γ2. The number of additional N-terminal amino acids in PPARγ2 *vs.* PPARγ1 is 28 in humans and 30 in rodents.

amino acids specific of γ2. On genomic DNA, this γ2-specific exon is located between the second mPPARγ1 exon (A2) and the first common exon (19, 24, 25). A third mRNA, PPARγ3, encodes the same protein as PPARγ1 but is controlled by an alternative promoter located in the region flanking exon A2 in 5′ (Fig. 1) (26).

B. DNA binding properties

The DBD is the most conserved domain between all nuclear receptors, and indeed is the hallmark of the superfamily. It is formed by two zinc finger-like motifs folded in a globular structure that can recognize a DNA target composed of 6 nucleotides. In most cases, nuclear hormone receptors bind as dimers to two copies of such a core motif, which constitute a functional hormone response element. The spacing of the two motifs and their relative orientation (*i.e.*, direct repeat, palindrome, or inverted palindrome configurations) determine which receptors bind to a given hormone response element (27). Members of the TR/RAR subfamily to which PPARs belong recognize preferentially the core hexanucleotide motif AGGTCA and are also characterized by the ability of forming a heterodimer with the 9-*cis*-retinoic acid receptor, RXR (NR2B). In fact, PPARs bind neither as homodimer nor as monomer but strictly depend on RXR as DNA-binding partner. Herein, the PPAR/RXR heterodimer will from now on be designated as PPAR:RXR.

1. PPAR response elements (PPREs) and PPAR:RXR binding properties. PPRE was first characterized by using synthetic oligonucleotides and was defined as a direct repeat of two core recognition motifs AGGTCA spaced by one nucleotide, thus also called DR1 (28). The first natural PPRE, found in the promoter of the acyl-CoA oxidase gene (15, 29), and all natural PPREs subsequently identified fulfill these DR1 criteria, which allow PPREs to be discriminated from other direct repeat response elements of the TR/RAR class of receptors, such as the one recognized by vitamin D receptor (VDR) (NR1I1) (DR3), TR (DR4), and RAR (DR2, DR5). However, the detailed analysis of the CYP4A6 and malic enzyme genes PPRE, together with a sequence comparison of 19 native PPREs and subsequent mutational analyses, defined additional PPRE determinants (30–33). The three following properties can be added to the initial PPRE definition as a DR1: an extended 5′-half-site, an imperfect core DR1, and an adenine as the spacing nucleotide between the two hexamers, giving the following consensus sequence PPRE: 5′-AACT AGGNCA A AGGTCA-3′. These particularities most likely add discriminating parameters that contribute to PPAR:RXR binding selectivity *vs.* homo- and heterodimers of other members of the superfamily, some of which also recognize a DR1 type element (see below).

The PPRE structure as an extended direct repeat motif imposes a polarity to the bound heterodimer. PPAR interacts with the upstream extended core hexamer of the DR1, whereas RXR occupies the downstream motif (31, 34). This represents a reversed polarity as compared with VDR:RXR and TR:RXR bound to DR3 and DR4, respectively, where RXR occupies the upstream core hexamer of the direct repeat. This difference in binding polarity between PPAR:RXR and VDR:RXR or TR:RXR is, at least in part, determined by the 5′-extended half-site in the PPRE. Receptors binding as monomers, such as NGFI-B (NR4A1), ROR (NR1F), and RevErbAα, also require an AT-rich 5′-extended binding site (35). Interaction of these receptors with the 5′-flank is thought to involve the receptor region immediately C-terminal to the second zinc finger, called carboxy-terminal extension (CTE). While PPARs are unable to bind DNA as monomers, it has been demonstrated that the CTE region of PPARs in PPAR:RXR is indeed responsible for the recognition of the 5′-flank of the DR1 in PPREs (36). The inability of PPAR to bind as a monomer has been attributed to the N-terminal region since deletion of the A/B domain of PPARα allowed the truncated receptor to bind to a PPRE as a monomer. Limitation of the DNA binding capacity of PPAR

by its A/B domain might reflect evolutionary changes that allow PPAR to functionally diverge from its monomeric cousins (36). While the three-dimensional structure of the DBD and CTE region of PPAR has not yet been solved, some of its properties can be inferred from detailed biochemical studies and structural analyses of RAR:RXR and TR:RXR bound to direct repeat sequences (37–45). Structural and biochemical analyses of RAR:RXR bound to a DR1 element demonstrate that the crucial amino acids for heterodimerization within the 5′-positioned receptor (RAR) are located in the second zinc finger, outside the first knuckle called D box, while the 3′-positioned receptor (RXR) contributes to the dimerization interface via its CTE region. Exchanging the specific PPAR D box, which has only three amino acids instead of five in other members of the superfamily, with that of RXR did not alter PPAR:RXR binding to a PPRE (G. Krey and W. Wahli, unpublished observations), consistent with the exclusion of the D box of the 5′-positioned receptor from the dimerization interface. The recent crystal structure analyses of the Rev-Erb DBD dimer bound on a DR2 (Rev-DR2) further confirm the presence of bonds between the tip of the second zinc finger of the upstream receptor and the GRIP box (VRF*GRIP*K residues) contained in the CTE region of the downstream receptor (46). Interestingly, PPARα, which possesses the same GRIP box as Rev-Erb, is also capable of binding as PPAR:RXR on a DR2 if the spacing sequence between the half-sites corresponds to that found in Rev-DR2 (AGGTCATCAGGTCA) in opposition to an alternative DR2 (AGGTCAGGAGGTCA) to which it does not bind. Furthermore, transcriptional activation by PPAR:RXR can be obtained through the Rev-DR2 that contains the conserved 5′-extended sequence which is recognized by dimers of Rev-ErbA and RORα (47). Therefore, a possibility of cross-talk exists between PPAR:RXR and these receptors on 5′-extended DR2 elements (36). The polarity of PPAR:RXR on such elements and the functional consequences of the formation of this complex have not yet been evaluated.

The fact that some tissues express more than one PPAR isotype raises the question of PPAR isotype-specific PPRE recognition. Assessment of the relative DNA-binding capabilities of the three PPAR isotypes (α, β, γ) to 16 native PPREs led to the classification of PPREs into three functional groups: strong, intermediate, and weak elements, which correlates with the level of the PPRE conformity to the consensus element (32). Surprisingly, the number of identical nucleotides in the core DR1 region is rather homogeneous across the different elements, and it is mainly the number of identities of the 5′-flanking nucleotides, rather than that of the stricto-sensu core DR1, which determines the binding strength of a given PPRE. In all cases, PPARγ binds more strongly than do PPARα and PPARβ and is thus less dependent on a well conserved 5′-flanking extension. In contrast, conservation of the 5′-flank is particularly essential for PPARα binding and therefore contributes to isotype specificity. The PPAR DNA-binding activity is also modulated by the isotype of the RXR heterodimeric partner. Binding of PPAR:RXR to strong elements is reinforced when RXRγ is the partner, whereas heterodimerization with RXRα is more favorable for binding to weak elements. However, it remains to be seen how these *in vitro* observations translate into selective recognition of the PPREs within their natural genomic and chromosomal environment.

2. Hormonal cross-talk occurring at the level of DNA binding. Direct repeat elements with a 1-bp spacing are also recognized by RAR:RXR, as well as RXR, androgen receptor-related protein-1, hepatocyte nuclear factor 4 (HNF-4) (NR2A), and chicken ovalbumin upstream promoter-transcription factor (NR2F) homodimers (48, 49). Accordingly, HNF-4 and chicken ovalbumin upstream promoter-transcription factor homodimers can displace PPAR:RXR from its binding site and thus compete with PPAR signaling (50–53). Evaluation of the biological significance of the competition between PPAR and other members of the superfamily for binding to PPRE requires the consideration of at least two parameters. First, the subtle sequence determinants that we described above are important for nuclear receptor discrimination. A recent study shows that single point mutations applied to the core DR1 motif differently affect the binding affinity of HNF-4, androgen receptor-related protein-1, RAR:RXR, and PPAR:RXR (54). Thus, the conjunction of a core recognition motif that deviates from the consensus with a specific 5′-flanking sequence, as seen in many natural PPREs, may result in preferential binding of PPAR:RXR (30, 31). Second, the relative amount of each type and isotype of nuclear receptor within a cell is of great importance in such a cross-talk.

Functional PPREs are almost exclusively represented by DR1-like elements. In addition to binding to the Rev-DR2 dicussed above, another exception is the ability of PPAR:RXR to recognize an estrogen response element (ERE) (55). Although an ERE-containing reporter plasmid can be transactivated by PPAR:RXR, no natural ERE-containing gene has been identified that is coactivated by estrogen receptor (ER) (NR3A) and PPAR:RXR. On the contrary, competition for the ERE leads to a PPAR:RXR-dependent repression of the ER-mediated transactivation of the vitellogenin gene A2 promoter as seen in transfection experiments. Thus the possibility of a hormonal cross-talk through an ERE exists (55, 56), and genes might be found to be coregulated by ER and PPAR:RXR, and consequently by estrogens, fatty acids, and 9-*cis*-RA, in a natural cell physiological context and in a promoter- and cell type-specific manner.

RXR is a common DNA binding partner to many nuclear receptors of the steroid/thyroid receptor superfamily, including PPAR. Consequently, competition between these receptors for their common partner can occur. Reciprocal negative interactions between the PPAR and TR signaling pathways, through a mechanism of RXR sequestration, was indeed demonstrated in transfection assays (57, 58). In these experiments, the relative amount of PPARs and TRs, respectively, determined which receptor was dominant, i.e., which signaling pathway inhibited the other. *In vivo*, such competition is likely to occur only when the amounts of RXR are limiting. Whether the relative amounts of TR, PPARs, and RXR meet these conditions in any tissue *in vivo* is so far unknown. A similar competition has been proposed to occur among PPAR isotypes (59). As a consequence, if several PPAR isotypes are coexpressed in a single cell type and if

RXR amounts are limiting, there is a possibility of differential activity of the expressed isotypes.

Interestingly, it was observed that expression of RXR abolished PPARα stimulation of the PRL promoter in pituitary GH4C1 cells (60). Analysis of this phenomenon revealed that stimulation of the PRL promoter by PPARα was mediated by protein-protein interaction rather than binding of PPAR:RXR to the promoter. The mechanism proposed is a ligand-dependent association of PPARα with the transcription factor GHF-1, which stimulates transcription and implies that PPARα would act similarly to a coactivator in this specific situation. Overexpression of RXR is thought to titrate out PPARα and therefore suppress its association with GHF-1 and consequently its stimulatory effect.

C. PPAR ligand-binding properties

One of the reasons for the present infatuation for PPARs lies in their particular ligand binding properties, making them attractive therapeutic targets. As we describe below, PPAR moved from the status of orphan receptor to that of generous host, capable of specifically interacting with more than one ligand, including some important natural compounds such as fatty acids. This section will end with information gained from the x-ray crystal structure of the PPARγ and PPARβ LBD, which provides a link between structural and functional viewpoints.

1. Tools for PPAR ligand identification. The first molecules able to activate PPAR were identified in cultured cells cotransfected with a GRE- or ERE-containing reporter gene together with an expression vector encoding the chimeric receptor GR_{DBD}-$PPAR_{LBD}$ or ER_{DBD}-$PPAR_{LBD}$. Compounds that trigger a stimulation of the reporter gene expression when added to the culture medium have been categorized as PPAR activators; the first identified were the fibrate hypolipidemic agents known to induce peroxisome proliferation in rodents, followed soon after by fatty acids (5, 10, 15, 61). However, since activation might result from indirect events such as production of a metabolite of the test compound, release of an endogenous ligand, or activation of a cell surface-initiated signaling pathway, these compounds had to be tested further for direct binding to the PPARs.

As could be anticipated from transactivation assays, classical competition assays using radioligands first identified some PPARα and PPARγ ligands with a relatively broad structural diversity (62–67). Additional techniques have then been adapted or developed to allow the screening of a large number of compounds (for review and technical aspects, see Ref. 68). The Scintillation Proximity Assay (SPA) is an equilibrium method that uses scintillation to measure the interaction between a molecule prebound to a fluomicrosphere and a radioactive ligand (69). It has been recently adapted to the evaluation of PPAR ligands in competition assays (70). The Differential Protease Sensitivity assay (DPSA) relies on a ligand-dependent reduction of PPAR sensitivity to enzymatic proteolytic cleavage (71). The Ligand Induced Complex (LIC) assay detects ligand-dependent binding of limiting amounts of PPAR:RXR to a PPRE (72). Based on the hypothesis that ligand binding to PPAR would induce interaction of the receptor with transcriptional activators, we have developed a novel sensor assay, termed Coactivator-Dependent Receptor Ligand Assay (CARLA) in which we measure the ability of a compound to induce PPAR-SRC1 interaction (73). Because of its strong interaction with PPARs, p300/CBP (cAMP response element-binding protein) can also be used in CARLA (74). In addition to ligand identification, these assays revealed three peculiarities of PPAR ligand binding properties that have important consequences for PPAR biology. First, in contrast to TR, RAR, VDR, ERα, or GR (glucocorticoid receptor) (NR3C1), PPARs accommodate several types of ligand, and the above-mentioned *in vitro* assays have demonstrated that most of the known PPAR activators are *bona fide* ligands (75). Second, and as a corollary, most of the molecules that specifically bind to PPAR do so with a rather low affinity as compared with the affinity of classical hormones for their cognate receptor. Third, there is some overlap in ligand recognition by the different PPAR isotypes, some ligands binding to more than one isotype although with different affinities. Although the known natural PPAR ligands fit well in our present understanding of PPAR functions, the question remains open whether, in addition, highly selective natural ligands exist with much higher affinity for each of the PPAR isotypes. Below is a presentation of the main PPAR ligands, natural and synthetic, discovered so far.

2. PPARα, PPARβ, and PPARγ ligands. The identification of unsaturated fatty acids as PPAR ligands (Table IA) provides firm evidence that at least part of the PPAR-dependent transcriptional activity of fatty acids results from a direct interaction of the nuclear receptor with these molecules. These fatty acids bind all three PPARs, with PPARα exhibiting the highest affinity, at concentrations that are in agreement with their circulating blood levels. In contrast, the very long chain fatty acid, erucic acid (C22:1), which is a weak ligand, appears more selective for hPPARβ than for hPPARα and hPPARγ, as measured in transfection assays using chimeric GR-PPAR proteins (76). Compared with the unsaturated fatty acids, saturated fatty acids are poor PPAR ligands in general (66, 72, 73), whereas phytanic acid, a dietary branched-chain, isoprenoid-derived fatty acid, efficiently binds PPARα (77). The discovery that some fatty acids can act as hormones that control the activity of transcription factors demonstrated for the first time that fatty acids are not merely passive energy-providing molecules but are also metabolic regulators. This finding opens novel perspectives for deeper understanding of energy metabolism and therapeutic interventions. Future investigations examining the differential tissue distribution of PPAR isotypes with respect to qualitative and quantitative fatty acid content of each tissue might be very informative for further understanding the specific roles of PPAR isotypes and their fatty acid-mediated activation.

Eicosanoids are a class of fatty acids mainly derived from arachidonic acid, either via the lipoxygenase pathway leading to the formation of leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) or via the cyclooxygenase pathway producing prostaglandins (PGs). Several of these eicosanoids are activators of the different PPARs, and some

TABLE 1. PPAR ligands

A. Natural ligands	PPARα				PPARβ				PPARγ			
	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA
Unsaturated fatty acids												
ω3-PUFAs												
α-Linolenic C18:3	+	+	+		+/−	+/−	+		+		+	
γ-Linolenic C18:3	+	+			+				+			
Eicosapentaenoic C20:5 (EPA)	+	+	+		+	+/−	+		+		+	
Docohexaenoic C22:6 (DHA)		+	+			+/−	−				+	
ω6-PUFAs												
Linoleic C18:2	+	+	+		+/−	+	+		+		+	
Dihomo-γ-linolenic C20:3	+				+				+			
Arachidonic C20:4	+	+	+		+	+			+		+	
ω9-mUFAs												
Palmitoleic C16:1	+				+				+			
Oleic C18:1	+		+		+		−/+		+		−	
Elaidic C18:1trans							−/+				−	
Erucic C22:1	−	−	−/+		−		−/+		−		−	
Nervonic C24:1		−	−				−/+				−	
ω2-mUFA												
Petroselinic C18:1	+		+				−/+		+		−	
Saturated fatty acids												
Capric C10:0	−				−				−			
Lauric C12:0	−	+/−			−				−			
Myristic C14:0	+	+/−			−/+				−/+			
Palmitic C16:0	+	+			+				−			
Stearic C18:0	+				+				−			
Arachidic C20:0	−				−				−			
Behenic C22:0	−				−				−			
Dicarboxylic fatty acids												
Dodecanedioic C12			−				−				−	
Eicosanoids												
±8-HEPE (hydroxyeicosapentaenoic)		+										
±8-HETE		+					−					
8S-HETE	+	+	+				+/−			−/+	−/+	
8R-HETE	−	−	+/−				−			−	−	
8(9)-EpEtrE		−										
±8-HETrE (hydroxyeicosatrienoic)		−/+										
12-HETE		−										
Leukotriene B4		−	+									
9-HODE (9-hydroxyoctadenoic acid)									+			
13-HODE									+			
15-Deoxy-Δ12, 14-PGJ2		−	−			−	−		+	+	+	

B. Synthetic ligands	PPARα				PPARβ				PPARγ			
	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA
Prostaglandin 12 analogs												
Carbaprostacyclin (cPGI)		+				+						
Iloprost		+				+						
Cicaprost		−				−						
Leukotriene B4 analogs												
Trifluoromethyl leukotriene B4			+									
ZK 151657			+				+/−				−	
ZK 158252			+				−				−	
Leukotriene D4 antagonist												
Ly 171883		+		+								
Hypolipidemic agents												
Clofibric acid		+	+	+		−						
Ciprofibric acid		+	−			−	−				+	
Bezafibric acid (xPPARs)			−				+				−	
Fenofibric acid			−				−				−	
Pirinixic acid (Wy-14643)		+	+			−	−				−	
GW 2331 (fibrate analog)	+								+			
GW 2433 (fibrate analog)	+				+							
Eicosatetraynoic acid (ETYA; arachidonic acid analog)			+	+			+				−	

TABLE 1. *Continued*

B. Synthetic ligands	PPARα				PPARβ				PPARγ			
	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA
Hypoglycemic agents (thiazolidinediones)												
Rosiglitazone (BRL 49653)	−	−	−			−	−		+		+	+
AD-5075									+			+
Troglitazone (CS-045)									+			
Hypolipidemic and hypoglycemic agents (nonthiazolidinedione)												
L-165041					+				−/+			
L-165461					+				+/−			
L-783483					+				+/−			
L-796449					+				+			
Nonsteroidal antiinflammatory drugs (NSAIDs)												
Indomethacin									+			
Flufenamic acid									+			
Fenoprofen									+			
Ibuprofen									+			
Carnitine palmitoyl transferase I (CPT1) inhibitors												
LY-171883		+		+								
2-Bromopalmitate (2Br-C16)		+										
Tetradecylglycidic acid (TDGA)		+										
Fatty acyl-CoA dehydrogenase inhibitors												
Ortylthiopropionic acid (OTP)		+										
Tetradecylthiopropinic acid (TTP)		+										
Nonylthioacetic acid (NTA)		+										
Tetradecylthioacetic acid (TTA)		+										

Direct interaction between natural (A) and synthetic (B) compounds and PPARs was analyzed by the following approaches: saturation binding assay (SABA), competition binding assay (COBA), PPAR:RXR-DNA ligand-induced complex formation (LIC), coactivator-dependent receptor ligand assay (CARLA), and differential protease sensitivity assay (DPSA). +, Binding detected easily; +/−, weak binding; −/+, very weak binding detected; −, compound tested but no binding detected. Absence of any sign means that the compound has not been tested with the corresponding method. The results summarized in the table are taken from Refs. (62, 64, 66, 71–73, 90, and 342). First results obtained with an additional technique, the fluorescence spectroscopy, are indicated in the text (82, 134).

are indeed ligands (see Ref. 78). 15-Deoxy-Δ12,14-PGJ2, which is a PGD2 derivative, is a ligand for PPARγ (63, 64, 73) and 8(S)-HETE, a compound associated with phorbol ester-induced inflammation, is a ligand for PPARα, whereas the 8(R)-isomer shows a much weaker binding (66, 72, 73). Leukotriene B4 (LTB4), a chemotactic inflammation mediator, binds *Xenopus* PPARα in classical binding assays, in CARLAs, and in LIC assays (65, 73, 79). Because binding affinities of molecules such as 8(S)-HETE and LTB4 for PPARα are in the submicromolar range, or micromolar range for fatty acids, one might dismiss at first glance these interactions as it would seem unlikely that tissue concentrations of these ligands can reach the levels required for receptor activation *in vivo*. However, the nuclear localization of 5-lipoxygenase in some cell types supports the idea that in the nucleus local concentrations of eicosanoids, such as LTB4, can reach high levels and that intranuclear action of endogenous leukotrienes is feasible (80, 81). Furthermore, the methods for assessing ligand dissociation constant (K_d) also deserve critical attention. The physical separation of bound and unbound molecules, often used in these techniques, leads to an equilibrium disturbance and often underestimates the K_d. To circumvent this problem, fluorescence-based methods that have been used to measure binding of retinoids to proteins are currently also applied to measure PPAR-ligand binding affinities (68). In this assay, the measurement of binding affinity is performed by optical means, which do not require the physical separation of bound and unbound molecules. This appoach has identified *cis*-parinaric acid as PPARγ ligand (82), and the affinity of many PPARα ligands, such as fatty acids and LTB4, is found to be in the nanomolar range in such analyses (83).

The CARLA as well as the DPSA and the LIC assays have proven effective tools for the identification of interesting synthetic compounds as ligands (Table 1B). These include some hypolipidemic agents such as fibrates, of which clofibrate and the potent Wy-14,643 compound preferentially bind PPARα. Thiazolidinediones (TZDs), which includes troglitazone, pioglitazone, and BRL 49653, now called rosiglitazone, are a class of antidiabetic drugs that are structurally derived from clofibric acid but selectively bind PPARγ (62, 84). This functional association of a key regulator of lipid metabolism and an antidiabetic drug has important implications with respect to the pharmacological use of these compounds, on the one hand, and for the link that it emphasizes between lipid and glucose metabolism on the other. This link is further reinforced by the discovery of a novel series of antihyperglycemic and antihyperlipidemic agents that are PPARγ agonists (85). Other synthetic compounds that bind to PPARs include the arachidonic acid analog

ETYA and some agonists and antagonists of the leukotriene membrane receptors (75). Intriguingly, the screenings for natural and synthetic ligands were not very successful in identifying PPARβ ligands. Bezafibrate is a *Xenopus* PPARβ-specific ligand (66, 73), but its activity is much weaker on the mammalian PPARβ. In transfection assays, the rat PPARβ can be activated by the nonmetabolizable, substituted fatty acid α-bromopalmitate. However, nonspecific toxic effects of α-bromopalmitate are found at doses close to those required for PPARβ activation (S. Basu-Modak, P. Escher, B. Desvergne, and W. Wahli, unpublished data). A novel series of fibrate derivatives, non-TZD compounds, was recently described as human-specific PPARβ agonist and will aid in the functional analyses of this elusive PPAR subtype (74).

The recent advent of the combinatorial chemistry technology opens new opportunities for the identification of PPAR ligands. Instead of relying on classical large and diverse compound libraries, Brown *et al.* (86) designed a solid phase synthesis of biased chemical libraries of fibrates (so-called focused library) based on the observation that fibrates have activity on the three PPARs. Screening of the library identified a pool of compounds with activity on each of the three PPAR isotypes, of which the compound GW 2433 exhibits a high, although not selective, affinity for xPPARβ. Thus, this approach may offer possibilities to develop selective and potent ligands for the three PPAR isotypes and has already provided a source of information about the ligand preferences of the three PPAR isotypes.

3. Species specificity in ligand recognition. Species difference in ligand recognition, already mentioned above for bezafibrate and xPPARβ *vs.* mammalian PPARβ, was first investigated with the PPARα isotypes from *Xenopus*, mouse, and human, which differentially respond to two PPARα ligands, Wy-14,643 and ETYA (67). Two amino acid residues in helix 3 of the LBD are responsible for the preferential responsiveness of *Xenopus* and hPPARα to ETYA and of mouse PPARα to Wy-14,643 (67). This identification of structure-function relationships involved in PPAR ligand binding specificity is of interest for drug development and may now be extended to additional compounds. These species differences, which have not been described for other nuclear receptors, raise two issues. The first, of practical importance, is that toxicological tests of PPAR ligands in whole animals must take into account possible species differences. The second is that the species-related ligand binding specificity may be linked to the speed of evolution of the PPAR genes. This might reflect an adaptation of the PPAR signaling pathways to nutritional patterns that can differ from species to species.

4. x-Ray crystal structure of the PPAR LBD. Although the LBD is less well conserved than the DBD between nuclear receptors, structural analyses of this domain performed with liganded RAR and TR LBDs and unliganded RXR LBD have revealed a common structural tridimensional fold, which consists of an antiparallel α-helical sandwich of 12 helices (helix 1 to helix 12) organized in three layers with a central hydrophobic pocket. Upon ligand binding, the swinging of helix 12 or activation function helix (HAF) closes the ligand binding pocket like a lid, in a so-called "mouse trap model" (87–89).

The x-ray crystal structure of the human apo-PPARγ LBD and apo-PPARβ reveals an overall fold very similar to that of the above mentioned LBDs from helix 3 to the C terminus (90–92). However, some distinct differences are apparent. The core AF-2 activation domain in the apo-PPARs is folded against the ligand binding pocket in a conformation similar to that observed in the holoforms of PPAR and other nuclear receptors. An additional helix, called helix 2′, which is found between the first β-strand and helix 3, together with a placement of helix 2 that differs from other nuclear receptor tertiary structures, provides an easy access to the hydrophobic pocket for ligands. The region between helix 2′ and helix 3, corresponding to the Ω loop in RAR is extended and is the most thermally mobile loop and participates in the structural changes occurring upon ligand binding. The ligand binding cavity is buried in the bottom half of the LBD and is particularly large, ≈1300 Å^3, of which the ligand occupies only about 30–40%. It is thus larger and more accessible than in other known LBDs [compare with the cavity in TR ≈ 600Å^3, most of this volume being occupied by the T_3 molecule ≈ 530 Å^3 (89)]. The T-shaped cavity in PPARγ comprised one region–the horizontal bar of the T–of 20 Å in length which lies parallel to helix 3; a second cavity region of 16 Å in length is orthogonal to the first and extends to the C-terminal AF2. The main amino acids involved in bonds with rosiglitazone are depicted in Fig. 2. Two histidine residues, H323 and H449, participate in the fixation of the TZD head group and are proposed to permit similar links with α-substituted carboxylic acids (91). The Y-shaped cavity in PPARβ comprises three arms of about 12 Å in length, the left arm being rather polar in character. Eicosapentaenoic acid occupies this pocket in two distinct conformations, with the acid group and eight first carbon units fitting in the left arm oriented toward the AF-2 helix, while the hydrophobic tail either bends upward or downward into the up or bottom arm of the pocket, respectively (90). The same network of hydrogen bonds as seen with roziglitazone and PPARγ forms between eicosapentaenoic acid and PPARβ AF-2 (see Fig. 2). These characteristics also explain that PPARβ ligands are preferentially unsaturated fatty acid, given the requirement of a flexible hydrocarbon tail, and have an optimal length, long enough for sufficient stabilizing hydrophobic interactions and short enough for being docked inside the cavity (90). In conclusion, these key interactions associated with the relatively free non-specific interactions that the hydrophobic part of ligands can develop within the large cavity explain the promiscuous behavior of PPAR with respect to ligand binding. One consequence might be that different functional activities of a ligand might reflect different binding conformation in the cavity (90). Together with the easy access provided by the extra helix 2′ and the tertiary placement of helix 2, these characteristics define PPAR as a nuclear receptor that has evolved to bind to multiple natural ligands with relatively low affinity, as reported above (91).

Taking into account the position and nature of the key residues bridging hPPARγ and its ligand, it is of interest to relate the effects of already described substitutions/mutations to these structural features (see Fig. 2). In PPARγ, four amino acids, namely aspartic acid 243 (D243) at the N terminus of the first β-sheet, arginine 288 (R288), glutamic acid

291 (E291), and glutamic acid 295 (E295) in helix 3, are determinants of the ligand entry site (91). E291 and E295 are conserved in all known PPARs, whereas D243 and R288 are only conserved in the γ-isotype, suggesting that these latter positions might be involved in isotype- and species-specific ligand selectivity. It has been observed that an experimental mutation of mPPARα in which E282 (marked with a *vertical line* in Fig. 2B), which corresponds to E291 in PPARγ, is replaced by a glycine results in a 4-fold loss of PPARα transcriptional response to Wy-14,643 and ETYA (93). *Xenopus*, human, and mouse PPARα respond differently to these two compounds. Two amino acids in helix 3 determine the preference for ETYA in the *Xenopus* and human receptor, isoleucine 272 (I272) and threonine 279 (T279), whereas these two positions (marked by *vertical lines* in Fig. 2B) are occupied by phenylalanine (F272) and methionine (M279) in the mouse receptor, which has a preference for Wy-14,643 (67). After substitution in the mouse receptor of F to I and M to T, the mouse receptor loses its preference for Wy-14,643 in favor of ETYA. Interestingly, M279 or T279 in PPARα corresponds to R288 in PPARγ, which supports the idea of a role of this position for ligand selectivity.

Finally, the crystal structure also revealed coiled-coil interaction between helix 10 from two PPARγ molecules forming a homodimer, reinforced by salt bridges involving helices 9 and 10. While PPARγ homodimers do not seem to occur *in vivo*, these observations are similar to those described for RXR homodimers (87) and are likely to reflect the contacts involved in PPAR:RXR. Consistent with these observations, a deletion comprising helix 10 and HAF of PPARα impairs heterodimerization with RXRα (71). Similarly, a leucine to arginine substitution at position 433 (marked by a *vertical line* in Fig. 2B) in helix 10 of PPARα also abolishes heterodimerization with RXR (58).

D. Alternative pathways for PPAR activation

1. PPARα and PPARγ are phosphoproteins. Several nuclear hormone receptors, including PPARs, are regulated by phosphorylation in addition to ligand-dependent activation. PPARα was first shown to be a phosphoprotein in primary rat adipocytes in culture. Treatment of these cells with insulin increases PPARα phosphorylation. In parallel, transfection studies in CV-1 cells and HepG2 cells revealed that insulin increases by nearly 2-fold the transcriptional activity of PPARα, as well as that of PPARγ (94). This insulin effect occurs through the phosphorylation of two microtubule-associated protein (MAP) kinase sites, identified at positions 12 and 21 in the A/B domain of hPPARα (95). Using cotransfections in a Chinese hamster ovary cell line that expresses the insulin receptor, Zhang *et al.* (96) demonstrated a synergistic effect between insulin treatment and PPARγ ligand-dependent activation on the expression of the target gene aP2. Because this effect was partially inhibited by the addition to the culture medium of a MAP kinase inhibitor, the insulin effect was correlated with PPARγ phosphorylation observed *in vitro* upon exposure to purified MAP kinase (96). Contrasting results were obtained when exploring the role of growth factors in modulating PPAR activity (97, 98). The epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) decrease the transcriptional activity of PPARγ while increasing PPAR phosphorylation through MAP kinase signaling. A unique MAP kinase target site, which can be used by both extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal kinase (JNK) (99), was mapped at serine 82 in the N-terminal domain of mPPARγ1, which corresponds to serine 112 of mPPARγ2. Substitution of this serine by alanine leads to a loss of PDGF-mediated repression of PPARγ activity (97, 98). Comparable MAP kinase-dependent PPARγ phosphorylation and inhibition of activity were obtained in 3T3-L1 cells with PGF2α, an arachidonic acid derivative that acts through a membrane receptor and has a potent inhibitory effect on adipogenesis (100). At the molecular level, the mutant PPARγ2 Ser112Asp exhibits a decreased ligand binding affinity and coactivator recruitment. Limited protease digestion of this mutant also results in an altered digestion pattern as compared with that of the native form of PPARγ2, when performed in the absence of ligands. These differences suggest that the N terminus, more specifically the phosphorylation status of the serine 112, plays a role in the conformation of the unliganded receptor, thereby regulating the affinity of PPARγ for its ligands (101).

How the same changes in phosphorylation can lead to an activation or an inhibition of PPARγ signaling, depending upon the nature of the triggering signal, insulin or growth factor, respectively, is unclear but likely involves either specific pleiotropic actions or use of different kinase pathways by these hormones acting on metabolic processes. For example, the insulin-mediated up-regulation of phosphoenolpyruvate carboxykinase (PEPCK) gene expression is independent of the Ras/MAP kinase pathway but relies on phosphatidylinositol 3-kinase (102). Another example is the inhibition of PPARα activity by GH through the Janus kinase 2-signal transducer and activator of transcription 5b (JAK2-STAT5b) pathway. This inhibition requires a nuclear and transcriptionnally active STAT5b molecule and likely occurs via an indirect mechanism (103).

Thus, the remaining open questions concerning PPAR phosphorylation emphasize the importance that posttranslational site-specific modifications may have in the cross-talk between cell membrane signaling and nuclear effectors.

2. Activation of PPAR:RXR by RXR agonists. Another alternative activation pathway of PPAR:RXR occurs through ligand binding to RXR. PPAR forms a permissive heterodimer with RXR, meaning that either partner can regulate the transcriptional activity of the DNA-bound complex by interacting with its cognate ligand, on its own or when both partners are liganded. Indeed, cotransfection studies have shown that both members of the PPAR:RXR complex can mediate a response in the presence of their respective ligand. Furthermore, cotreatment of the cells with both ligands results in an additive effect. Thus, the natural RXR ligand 9-*cis*-RA as well as synthetic RXR-selective compounds such as LG 1069 and LG 100268 can activate a PPRE-driven reporter gene in a PPAR:RXR-dependent manner (28, 61, 104). Other examples are the liver fatty acid binding protein (L-FABP) gene and the ApoAII gene, known PPAR target genes, which are also responsive to 9-*cis*-RA (105, 106). *In vivo*, this ability of PPAR:RXR to transduce 9-*cis*-RA signal has been associated with

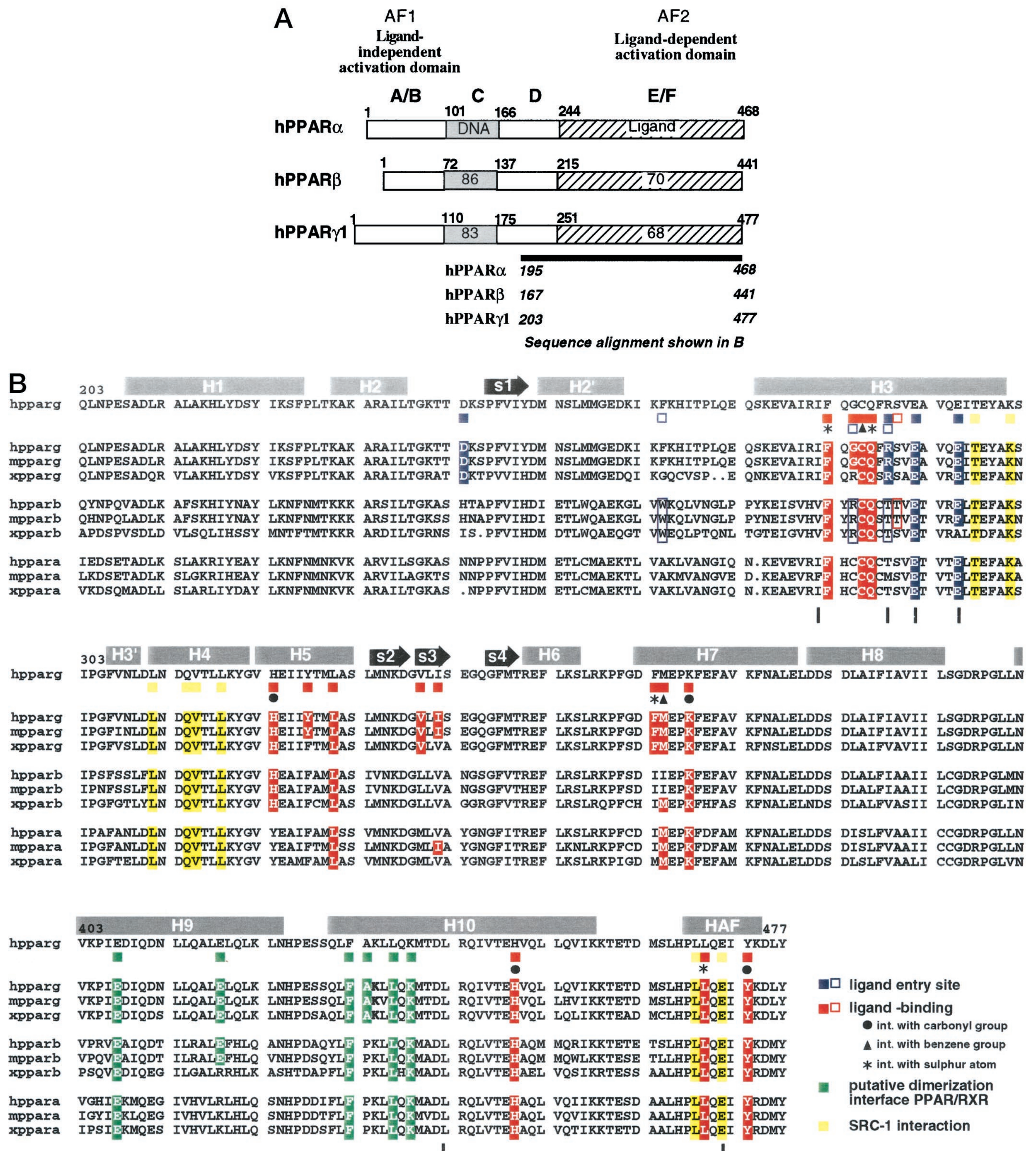

FIG. 2. General structure and LBD of PPARs. A, Scheme indicating the four-domain structure of PPARs, which is the same as for the other members of the nuclear receptor superfamily. Coordinates of the boundaries of each domain are given for the hPPARs. The *number inside each domain* corresponds to the percentage of amino acid sequence identity of human PPARβ and PPARγ relative to PPARα. The region whose amino acid sequence is shown in panel B is *underlined*, and the coordinates are according to the following Genbank accession numbers: PPARα, L02932; PPARβ, L05792; PPARγ, X90563. DNA indicates DBD; ligand indicates LBD; aa, amino acids. B, Sequence alignment of the human (h), mouse (m), and *Xenopus* (x) LBD of PPARγ (PPARg), PPARβ (PPARb), and PPARα (PPARa). The secondary structure adopted by hPPARγ is indicated *above the sequence in boxes* for α-helices (H) and *arrows* for β-strands (s). Amino acids that are involved in determining the ligand entry site,

the observation that RXR-selective agonists display antidiabetic activities comparable to those obtained with a TZD, which specifically binds PPARγ (107). This *in vivo* action of 9-*cis*-RA is not PPARγ specific since it also activates PPARα-inducible genes (108). The physiological relevance in the whole organism of 9-*cis*-RA pathways has been recently underscored by the identification of two 9-*cis*-retinol dehydrogenases that might participate in the synthesis *in vivo* of 9-*cis*-RA (109, 110). However, interpretation of the above-mentioned results, obtained after 9-*cis*-RA stimulation in relation to the potential clinical use of RXR agonists, calls attention to the three following points: 1) in addition to acting through PPARγ:RXR and PPARα:RXR, RXR agonists *in vivo* might also recognize and act via NGFIB:RXR and LXR:RXR permissive heterodimers (111–113); PPARβ:RXR-mediated effects can also not be excluded; 2) in the absence of ligands, RXR forms inactive tetramers and addition of RXR-specific ligands preferentially directs the formation of homodimers rather than heterodimers (114). Thus, the formation of heterodimers, and the subsequent signaling, also depends on the presence of ligands for the heterodimerization partner (115); 3) RXR homodimers, whose formation is favored by 9-*cis*-RA, bind and transactivate through response elements corresponding to DR1 (48, 116). In the context of the PPAR:RXR signaling pathway, how much of the 9-*cis*-RA response is PPAR dependent and how much is relayed by the formation of RXR homodimers remain to be determined. Such a question is brought up, for example, by the apparent contradictory observations concerning apoCIII gene expression whose down-regulation by fibrates is mediated by PPARα (117), whereas the same gene is up-regulated by RXR-specific agonists (118). While little RXR homodimers are seen binding on PPREs *in vitro*, the situation *in vivo* remains to be explored. The use of a more comprehensive panel of RXR agonists, partial agonists, and antagonists should be used for investigating the complex network built up by RXR within nuclear receptor signaling. This is becoming possible with the recent characterization of the LG 100754 compound, which is a specific RXR:RXR antagonist but RAR:RXR and PPAR:RXR agonist (119, 120).

In contrast to its role in PPAR:RXR, RXR can only work in subordination when heterodimerized with RAR, *i.e.*, the liganded RXR is transcriptionally active only when RAR is itself liganded (Refs. 121–123 and references therein). A similar situation may apply for heterodimers between RXR and either TR or VDR. This would prevent retinoids from activating VDR or TR pathways, in contrast to that mediated by PPAR. It is not yet known whether these different transactivation properties reflect the different polarity with which PPAR:RXR, on the one hand, and RAR:RXR, TR:RXR, and VDR:RXR, on the other, bind to their respective response elements.

E. PPAR-mediated transactivation properties

PPAR-mediated transactivation results from the combination of PPAR:RXR binding to a PPRE and ligand activation of this complex. The conformational change of PPAR triggered by ligand binding or by other activation processes, such as phosphorylation, is believed to generate a conformation with new protein-protein interacting surfaces, that will allow specific contacts with a coactivator(s). Subsequently, this complex transduces regulatory action to the basal transcriptional machinery (see Fig. 3).

1. Delineation of activation domains in PPARs. The ligand-dependent activation domain, called AF2, is found in the LBD and is only transcriptionally active in response to ligand binding. Sequence alignment and mutation analyses have helped to locate a potent core activation domain at the very C terminus of the xPPARα LBD. This region has some amphipathic helix characteristics and consists of two overlapping motifs, I and II, containing the core sequence ΦXE/D$\Phi\Phi$ (Φ represents hydrophobic residues and X represents residues with long side chains). Motif II terminates at the last residue of the receptor C terminus while motif I terminates four residues before the C terminus. Both motifs are important for PPAR ligand-dependent transcriptional activity, and motif II might act indirectly by stabilizing an optimal conformation of motif I (G. Krey, A. Hihi, and W. Wahli, unpublished data).

While the ligand-mediated activity of PPAR is not affected by the deletion of its A/B domain, a ligand-independent activation function AF1 has been defined within this domain, for both PPARγ and PPARα. The PPARγ AF1 activity was assayed in transfection analyses using chimeric transcription factors fusing the Gal4DBD with the PPARγ1 or PPARγ2 A/B domain. While both A/B domains are active, the mPPARγ2 N terminus, which possesses an extra 30 N-terminal amino acids, is 5- to 10-fold more potent in transcriptional activation than the mPPARγ1 N terminus (124). Interestingly, mutation of the MAP kinase site at serine 82/112 described above precludes phosphorylation of this site and increases the activity of Gal4-PPARγ AF1 (125), suggesting that phosphorylation at serine 82/112 not only affects ligand binding affinity as discussed above (101) but also directly regulates the AF1 activity. Similarly, hPPARγ2 with a proline-to-glutamine conversion at position 113 (given as position 115 in the original article), when overexpressed in murine fibroblasts, is defective in serine 112 phosphorylation and accelerates differentiation of the cells into adipocytes

ligand binding, dimerization, and interaction with SRC-1 are indicated by *colored filled boxes*, for hPPARγ as well as for the other PPARs when conserved. Residues involved in specific interactions with the carbonyl group, the benzene group, and the sulfur atom of rosiglitazone are indicated by a *black dot*, a *triangle*, and an *asterisk*, respectively. These data are derived from the x-ray crystal structure of the LBD of the hPPARγ isotype in a complex containing the ligand rosiglitazone (BRL 49653) and 88 amino acids of human SRC-1 (91). Results obtained from the crystal structure of the LBD of the hPPARβ isotype containing the fatty acid eicosapentaenoic are consistent with those of PPARγ (88). Individual residues for ligand entry and ligand binding to PPARβ are indicated by the corresponding *colored empty boxes*. The dimerization PPAR:RXR interface is postulated, taking into account the residues conserved in RXR that contribute to the interface of a PPAR:PPAR homodimer observed in crystal solution (91). The mutations previously characterized and cited in the text are indicated by *vertical lines* below the sequence of helix 3, helix 10, and HAF (helix containing the activation function 2 core).

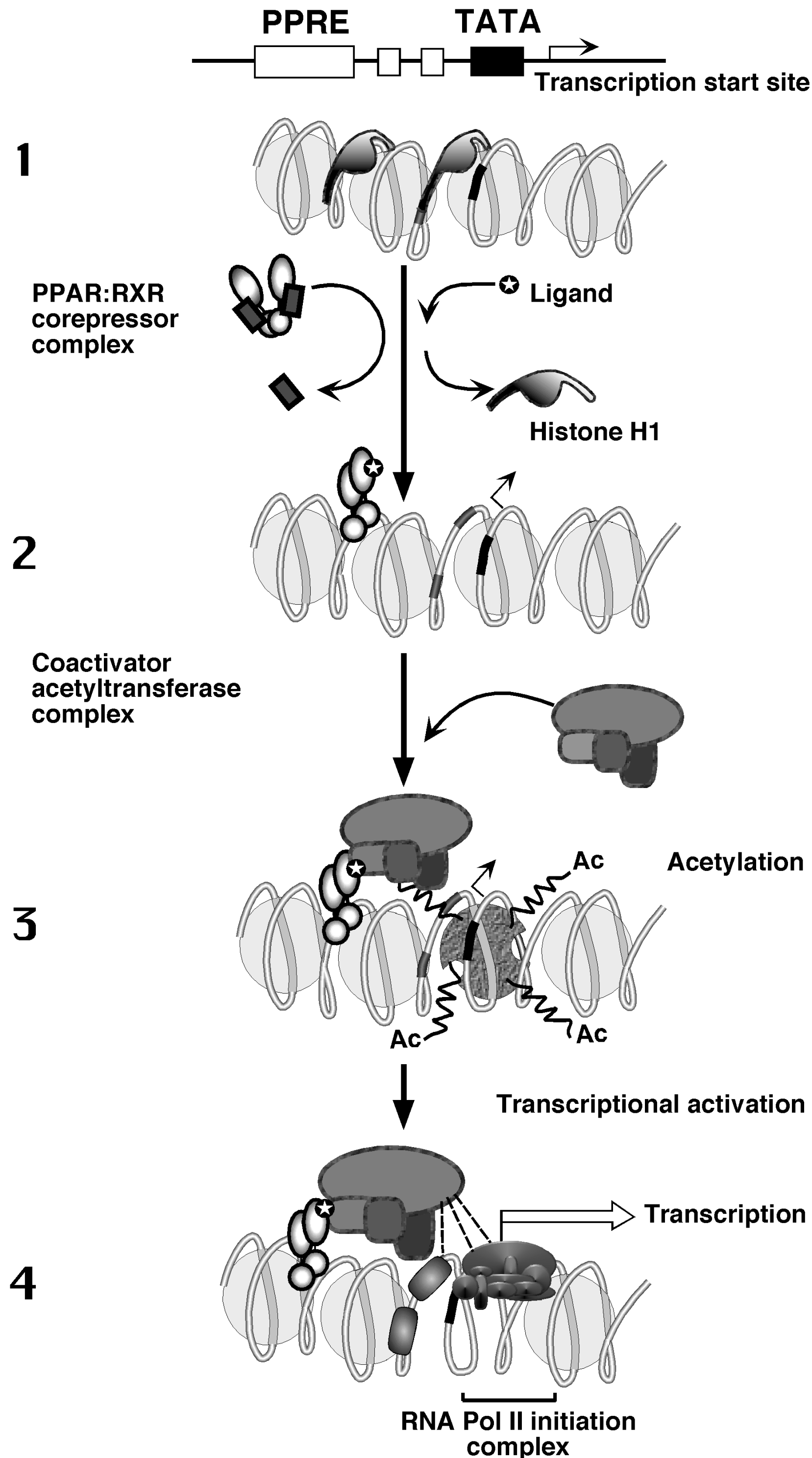

FIG. 3. Model for transcriptional activation by PPARs. 1, Scheme of a PPAR-responsive promoter presented in a linear form with a PPRE, two binding sites for transcription factors (*white box*), the TATA box, and the transcription start site. The same region organized in a repressive chromatin structure is also shown. A hypothetical PPAR:RXR/corepressor complex, which is not bound to DNA, is activated by a ligand that results in a dissociation of the corepressors from the ligand-activated PPAR:RXR complex. 2, The activated PPAR:RXR complex binds to the PPRE producing a change in chromatin structure indicated by histone H1 release. PPRE-bound PPAR:RXR targets a coactivator-acetyltransferase complex to the promoter. 3, The promoter chromatin at the transcription initiation site region is modified by the coactivator-acetyltransferase complex, which acetylates the histone tails (Ac) thereby producing a transcriptionally competent structure. Acetylation of histones is selectively enriched at the transcription initiation region, involving one to two nucleosomes. 4, Additional transcription factors (*e.g.*, Sp1, NF1) and the basal transcriptional machinery, including the RNA Pol II initiation complex, are recruited to the accessible promoter and transcription is initiated.

when compared with wild-type PPARγ2 (126). Insulin, in contrast, enhances the transcriptional activity of the PPARγ AF1 (124), as well as induces the activity of the PPARα AF-1 domain (95), suggesting that insulin also induces phosphorylation of PPAR via an alternative site or a mechanism that may involve posttranslational modification of an auxiliary factor. No regulated activity of the N-terminal domain of PPARβ has been reported so far.

2. PPAR interaction with cofactors. There is strong evidence for the crucial inhibitory or stimulatory role played by molecules that provide a bridge between DNA-bound transcription factors and the transcription initiation machinery (127, 128). The nuclear receptor corepressor (N-CoR) and the silencing mediator for retinoid and thyroid hormone receptor (SMRT) are corepressor proteins that interact with unliganded nuclear receptors, mediating a repressive signal to the promoter to which the complex binds. Interaction with corepressors requires the CoR box, a structural motif in the N-terminal part of the LBD, which has been described in TR and RAR. PPARγ interacts strongly with N-CoR and SMRT in solution but not when bound to a PPRE as a PPAR:RXR complex (34, 129). In association with the absence of a conserved CoR box in PPARs (34), these data provide an explanation for the absence of transcriptional repression by unliganded PPAR via its response element, in contrast to the repression activity of unliganded TR and RAR.

The first described nuclear receptor coactivator, steroid receptor coactivator 1 (SRC-1) (130), can interact with the PPAR LBD in solution. This interaction is ligand dependent, and we used this property to develop the CARLA screening assay (73). Two PPAR binding domains in SRC-1 have been identified (131). Furthermore, the liganded PPARγ LBD has been cocrystallized with the region of SRC-1 (aa 623–710) encompassing two LXXLL motifs (where X is any amino acid), characterized as consensus sequence found in nuclear receptor-associated factors (132). The x-ray crystal structure shows that in the presence of the ligand rosiglitazone, the two LXXLL motifs of a single SRC1$^{623-710}$ molecule interact separately with the AF2 helix (HAF, see Fig. 2B) of each receptor molecule of a dimer, making a stable ternary complex: two PPARγ LBDs and one SRC1 molecule (91). In this structure, the LXXLL helix is oriented by a conserved glutamic acid of HAF (E471, see Fig. 2B, *vertical line*) and a conserved lysine in helix 3 (K301) of the LBD, allowing the placement of the LXXLL coactivator motif into the hydrophobic pocket formed by helices 3, 4, 5, and HAF of PPARγ (93). Importantly, distinct amino acids C-terminal to the core LXXLL motif are required for PPARγ activation in response to different ligands (133). In agreement with this structure, a site-directed AF2 mutant of PPAR (E471A) that has lost transcriptional capability also fails to interact with SRC-1 or CBP (134). Functional assessment of the importance of SRC-1 in PPAR-mediated transactivation comes from the overexpression of the nuclear receptor-interacting domain of SRC-1, which inhibits PPAR-dependent transactivation, whereas overexpression of the full-length SRC-1 potentiates ligand-dependent transcription by PPARγ:RXR (34, 131). In addition, microinjection of SRC-1-directed antibodies inhibits the TZD-dependent activation of a PPRE reporter gene in Rat-1 cells, this inhibition being rescued by the coinjection of a plasmid expressing the full-length SRC-1 (135). In contrast, PPARα is still transcriptionally active in SRC-1 knock-out (KO) mice, suggesting that its activity can also be mediated through interaction with other nuclear receptor coactivators (136).

Some studies have indicated that liganded PPAR might preferentially directly interact with CBP or the related protein p300 (137–139). This preference for CBP compared with SRC-1 has been confirmed using fluorescence energy transfer as an approach for quantitating such interactions (134). CBP/p300s are very large proteins that are essential for the transactivation function of many transcription factors including nuclear receptors (140–142), AP-1 (143), cAMP response element-binding protein (143, 144), basic helix-loop-helix factors (145), STATs (146–148), and nuclear factor-κB (NF-κB) (149). Evidence from gene inactivation in mice and from the Rubinstein-Taybi syndrome in humans demonstrates that CBP/p300s are limiting factors (150). Thus, they are viewed as integrators of multiple signaling pathways, linking membrane receptor signaling and nuclear receptor activation pathways, as well as being a key limiting factor for which all the above mentioned pathways must compete. In parallel to these functional interferences between distinct pathways, the ability of CBP/p300 to simultaneously make contacts with more than one transcription factor might explain some synergy between these factors (127, 151, 152). Clarifying the specific contacts that PPAR may have with CBP/p300 is thus of great importance for understanding the molecular mechanism of the wide range of PPAR action. The first observations along this line showed that deletion of the 20 C-terminal amino acids of PPARα abolished interaction with CBP, whereas the mutated receptor still binds Wy-14,643, suggesting that like SRC-1, CBP interacts with the activation function helix of PPAR (137). However, the LBD of the receptor alone (amino acids 281–468) is not sufficient for a stable ligand-dependent interaction with CBP, which also requires the participation of the T box in the D domain (amino acids 166–179). It is noteworthy that CBP can make functional interactions with SRC-1 through leucine-rich motifs in SRC-1 different from those required for interaction with PPAR. Therefore, it becomes apparent that these motifs serve several functions that are likely to control receptor- and ligand-specific coactivator recruitment as well as the assembly of extended complexes required for the transcriptional induction of receptor target genes.

Schulman *et al.* (139) explored a complex mammalian hybrid system involving fusion proteins to decipher the molecular mechanism of the PPAR:RXR activation by RXR agonists through interaction with CBP. This study shows that the activation of the heterodimer through RXR ligand is independent of the RXR AF2 activation domain but rather involves a conformational change of RXR, which is propagated to the unliganded PPAR moiety and leads to a PPAR ligand-independent interaction of the PPAR AF2 domain with the cofactor CBP. However, the system used does not take into account the role of DNA as triggering some specific constraints to the PPAR:RXR complex. Using the same approach, this "phantom ligand effect" was also previously described for RAR:RXR (153). In contrast to PPAR:RXR, RAR:RXR cannot be made transcriptionally active by a RXR ligand alone. Moreover, the RXR AF2 domain is crucial for retinoid sig-

TABLE 2. Cofactors interacting with PPARs

	Described interactions with		Ref.
	PPARs	Other NR	
Coactivator			
SRC-1	α, β, γ	GR, TR, ER, PR, RXR, RAR, VDR	(34, 73, 91, 130, 131, 134, 135, 384–386)
CBP/p300	α, γ	AR, ER, RAR, VDR, PR, RXR, TR	(137–142, 387–389)
PGC-1	γ	RAR, ER	(155)
PGC-2	γ	ER	(390)
PBP/TRAP220/DRIP230	α, γ	TR, RAR, RXR, VDR	(156–158)
RIP 140	α, γ (ligand-independent inhibition of PPAR activity)	TR, RXR, TR2, LXR	(159, 160, 391)
ARA70	γ	AR	(392, 393)
Corepressor			
SMRT	γ	TR, RAR, VDR, COUP-TF	(34, 129, 159, 394–396)
N-CoR	γ, α	TR, COUP-TF, VDR, RAR, Rev-ErbA	(34, 386, 396–400)

Cofactors for which direct contacts with PPARs have been described are listed, together with the interaction of these cofactors with other nuclear receptors (NR). SRC-1, steroid receptor coactivator 1; CBP, CREB binding protein; P300, E1A-associated factor; PGC, PPARγ coactivator; PBP, PPAR-binding protein; TRAP220, thyroid receptor-associated protein 220; DRIP230, vitamin D receptor interacting protein 230; RIP140, receptor interaction protein 140; ARA70, AR-associated protein 70; SMRT, silencing mediator for retinoid and thyroid hormone receptor; N-CoR, nuclear receptor corepressor.

naling since a mouse that bears a deletion mutation of the RXRα AF2 domain dies around birth with most of the symptoms corresponding to vitamin A deficiency (154). Thus, while the findings described above are of great interest with regard to the PPAR:RXR mechanism of action, more experiments are required to integrate them into a physiological context.

Other PPAR interacting proteins, such as PPARγ coactivator 1 (PGC1), PGC2, PPAR-binding protein (PBP)/thyroid hormone receptor-associated protein 220 (TRAP220), receptor-interacting protein 140 (RIP140), and androgen receptor-associated protein 70, have recently been cloned (Table 2). PGC1 is a factor that can interact with PPAR (in a ligand-independent manner), TR and ER (in a ligand-independent manner, but with further reinforcement by thyroid hormone and estrogen, respectively), and RAR (in a ligand-dependent manner) (155). This factor is of particular interest because of the strong induction of its expression in muscle and brown fat upon cold exposure. Cotransfection assays indicate a major role of PGC1 in activating a brown fat-specific uncoupling protein 1 (UCP1)-reporter gene in the presence of PPARγ, RXRα, and a cocktail of ligands (troglitazone, 8-bromo-cAMP, and 9-*cis*-RA) (155). The ubiquitously expressed PPAR binding protein (PBP) (156), also cloned as the TR-associated protein TRAP220 (157) and VDR interacting protein DRIP230 (158), was identified in a double hybrid screen using the PPARγ LBD as bait. In a cotransfection assay, its overexpression slightly increases PPARγ activation while a truncated form has a potent dominant negative effect (156). This suggests that PPAR might also use the large multisubunit coactivator complex (DRIPs or TRAPs), which was shown to be anchored through the ligand-dependent interaction of DRIP230/TRAP220/PBP with VDR (158). Interaction of the PPAR LBD with RIP140, initially identified in a breast cancer cell line, is ligand independent (159). Interaction with the DNA-bound PPAR:RXR only occurs in the presence of 9-*cis*-RA and not in the presence of a PPAR ligand (160), suggesting that RIP140 interacts with the RXR moiety of the heterodimer rather than with PPAR. In transfection assays, the SRC1-mediated enhancement of PPARγ activity is down-regulated by increasing doses of RIP140 expressing plasmid, suggesting that RIP140 acts as competitor and inhibits SRC1 binding to PPAR:RXR (160).

In addition to cofactors, PPARs have been shown to functionally interact with at least one other transcription factor. For regulation of the acyl-CoA oxidase promoter, PPAR:RXR exerts its effect through two PPREs in synergy with the transcription factor Sp1 via five Sp1-binding sites (161). These and the above observations underscore that PPAR action at any particular time in the cell will depend on the availability of several transcription factors and cofactors as well as on stimuli that can affect the levels or activity of these transcriptional components.

In response to these interactions, coactivators and corepressors alter target promoter activities by a mechanism that associates chromatin modification–via their intrinsic histone acetyltransferase or deacetylase activity, respectively, as demonstrated for CBP/p300 (162–164)–and physical contact with the transcription initiation machinery (152) (Fig. 3). The fact that coactivators are shared by different nuclear receptors and other transcription factors may indicate that coactivators serve an important role in cell growth and differentiation as contributors to the selection of specific nuclear signaling pathways in a spatio-temporal manner. Assessing which and how a signal or biological situation would trigger preferential interaction of one or the other nuclear factor with PPAR is critical for understanding the integration of PPAR-mediated events in the complexity of the nuclear regulatory network.

III. Physiological Aspects

A. Differential expression of PPAR mRNAs

Information on PPAR expression patterns is a first step in understanding the biological significance of the existence of different PPAR isotypes and isoforms. PPAR isotypes are often coexpressed in tissues that are of ectodermal, mesodermal, or endodermal embryonic origin, with relative levels that vary from one cell type to the other. For the sake of clarity,

the expression patterns will be presented separately for each of the three PPAR isotypes in *Xenopus*, rodents, and human.

1. PPARα expression and regulation. In *Xenopus*, PPARα is expressed at moderate levels during oogenesis. The maternal transcripts persist in the early embryo up to gastrula stages (15). They are then replaced by zygotic transcripts at tail bud stage. In the adult, PPARα is expressed in all tissues that have been tested, *i.e.*, liver, kidney, muscle, testes, and fat body. In mouse and rat, PPARα appears relatively late in development (E13.5) in the tissues where it will be expressed in adulthood. In addition, there is a transient expression of PPARα in the developping central nervous system and during skin maturation (8, 165, 166). In the adult rat, relatively high levels of PPARα mRNA are detected in brown fat, liver, kidney, heart, and the mucosa of stomach and duodenum. Retina, adrenal gland, skeletal muscle, and pancreatic islets also express significant amounts of PPARα mRNA (167, 168). In the human, its levels in the liver appear lower than in the rodent liver (169). In addition, a splice variant lacking exon 6 is found, in addition to the full-length mRNA, in all human liver samples examined (169). Because of a shift in the open reading frame, the resulting protein is truncated shortly after the DBD, but no functional analyses of this short form is presently available. Other data available in the human indicate that PPARα is well expressed in heart, kidney, skeletal muscle, and large intestine (170, 171). In summary, and regardless of the species, the expression of PPARα correlates with high mitochondrial and peroxisomal β-oxidation activities, as exemplified by its high levels in cardiomyocytes and cells of the kidney proximal tubules, which primarily use fatty acids as an energy source. Another example is the enterocytes at the top of the intestinal villi, which carry the main burden of fatty acid absorption and have a very active peroxisomal β-oxidation.

In rat liver, PPARα expression is subject to negative and positive regulation by insulin and glucocorticoids, respectively (172, 173). Accordingly, PPARα mRNA and protein levels cycle in parallel with the circadian rhythm of circulating glucocorticoids. Stress situations or fasting, which induce the levels of plasma glucocorticoids, also result in increased synthesis of PPARα in hepatocytes (174, 175). In contrast, exposure of primary culture of rat hepatocytes to GH for several days decreases PPARα mRNA levels by 50%. This suppression of PPAR expression may participate in the inhibition of peroxisome proliferator-induced peroxisomal β-oxidation by GH (176). A down-regulation of PPARα gene expression was also observed in chronic alcoholic liver disease in the rat (177). Finally, an up-regulation of PPARα by its own ligands, fibrates, or FFA has been found in the FaO rat hepatoma cell line and in rat pancreatic islets, but whether the regulation is transcriptional or posttranscriptional remains to be clarified (178, 179).

2. PPARβ expression and regulation. In *Xenopus*, PPARβ also accumulates early during oogenesis and is expressed in oocytes even at higher levels than PPARα (15, 180). These maternal transcripts slowly disappear in the early embryo up to gastrula stages to be replaced by zygotic transcripts at the neurula stage. In the adult *Xenopus*, PPARβ expression is ubiquitous, with varying levels in different organs. During rat development, it is already present at relatively high levels in embryonic ectoderm and visceral and parietal endoderm at stage E8.5. The expression shows an important peak in the neural tube between E13.5 and E18.5, and then remains ubiquitous at a lower level throughout the end of development (165, 166). At the adult stage, PPARβ is also abundantly and ubiquitously expressed, often at higher levels than PPARα and PPARγ. It also remains the most expressed isotype in the adult nervous system (165, 166, 181). It is only weakly expressed in liver, as compared with other tissues such as lung and kidney (4, 8, 167). Although it is abundant in skeletal and cardiac muscle, PPARβ cannot be detected by *in situ* hybridization in smooth muscle cells of the digestive tract. In testis, its expression is very high in Sertoli cells (167). Interestingly, its expression is markedly induced in the uterus at the time of blastocyte implantation and remains abundantly expressed in the decidua at the postimplantation stage (182). In humans, PPARβ is present at moderate levels in all tissues tested, with a higher expression in the placenta and the large intestine (170, 171). So far, very little is known about the regulation of the PPARβ gene. In the ob1771, 3T3-L1, and 3T3-F442A adipose cell lines as well as in the myoblast cell line C2C12, levels of PPARβ transcripts appear to be low in proliferating cells and are induced upon differentiation (9).

3. PPARγ expression and regulation. In contrast to xPPARα and xPPARβ, xPPARγ mRNA is not detected during oogenesis in *Xenopus* except for a short transcript that does not encode a full-length receptor (15, 180). At adult stage, xPPARγ has a relatively restricted expression, with the highest levels found in the fat body and moderate levels found in kidney and liver. Similarly, PPARγ has a restricted pattern of expression in adult rodents, white and brown adipose tissues being the major sites of expression (183). The intestinal mucosa also express high levels of PPARγ in the colon and cecum but less in the small intestine (184–186). Strikingly, PPARγ is abundant in lymphoid tissues such as the spleen (red and white pulp) and Peyer's patches in the digestive tract (167, 168). Finally, PPARγ is also expressed at low levels in the retina and skeletal muscle. In humans, both PPARγ1 and γ2 are abundant in adipose tissue and are present at low levels in skeletal muscle. In addition, hPPARγ1 is also found in liver and heart (170, 187). In contrast to rodent, hPPARγ is detected neither in spleen nor in circulating T lymphocytes, whereas it is expressed in several transformed human B lymphocyte and myeloid cell lines, as well as in primary bone marrow stromal cells in culture (14). Intriguingly, circulating lymphocytes or polymorphonuclear cells express a short 0.65-kb PPARγ transcript of unknown function, similar to that found in *Xenopus* oocytes.

Regulation of the hPPARγ gene has been analyzed *in vitro* as well as *in vivo. In vitro* exposure of isolated human adipocytes to insulin and corticosteroids synergistically induce PPARγ mRNA (187). In contrast, PPARγ is down-regulated by tumor necrosis factor-α (TNFα), which triggers dedifferentiation of mature adipocytes in parallel to reducing the expression of adipocyte-specific genes (188, 189). Since PPARγ plays a key role in adipogenesis and is the receptor for insulin-sensitizing drugs, regulation of its expression

with respect to nutrition, obesity, and diabetes has been studied. In rodents, which produce a high-fat milk, the suckling-weaning transition of the young corresponds to a dramatic change in dietary fat. PPARγ2 is increased in white adipose tissue during suckling and the suckling-weaning transition but rapidly reaches a stable plateau (190). In adult rats fed by oral gavage for at least 4 days, PPARγ is significantly increased in the adipose tissue of rats receiving high-fat meals, but not in the animals receiving high-carbohydrate food (191), whereas 48 h of fasting dramatically reduces the expression of both PPARγ isoforms in subcutaneous and visceral adipose tissue (192). In mice, PPARγ gene expression is also regulated by nutrition and obesity. The expression of both isoforms is down-regulated by fasting and by insulin-deficient diabetes, whereas exposure to high-fat diet increases PPARγ expression in adipose tissue of normal mice and induces PPARγ2 expression in the liver of obese mice (193).

PPARγ expression was studied in subcutaneous adipose tissue of 14 lean and 24 obese subjects, revealing that adipose tissue of obese humans has increased expression of PPARγ2 mRNA, as well as an increased ratio of PPARγ2/γ1, in proportion to the body mass index (BMI) (187). In addition, a low-calorie diet specifically down-regulates the expression of PPARγ2 mRNA in adipose tissue of obese humans. Increased PPARγ2/γ has also been observed in obese rhesus monkeys (194). At the same time, a study involving 29 subjects with various degrees of obesity concluded that mRNA levels of PPARγ1 in abdominal subcutaneous adipose tissue do not correlate with BMI or fasting insulinemia (Ref. 171; see also Ref. 195). Together these results suggest that, in humans, PPARγ2 but not PPARγ1 is involved in the control of adipocyte function. This hypothesis provides a possible molecular mechanism for the alterations in obesity of adipocyte number and function but will require further large-scale studies to be validated. Expression of PPARγ has also been studied in muscle tissues and cultures from lean subjects, obese nondiabetic subjects, and patients suffering from type 2 diabetes mellitus. PPARγ (γ1 and γ2 not distinguished) was increased in both obese nondiabetic and type 2 diabetes mellitus patients in direct relation to BMI and fasting insulinemia, suggesting that abnormalities of PPARγ might be involved in skeletal muscle insulin resistance linked to obesity and diabetes (196). In addition to these quantitative analyses of expression levels, mutations in hPPARγ have been studied with respect to obesity and diabetes. A polymorphism affecting codon 12 of PPARγ2, substituting a proline to an alanine, has been found in several different populations, suggesting that it must be of ancient origin (197). This substitution has been associated with decreased receptor activity, lower BMI, and increased insulin sensitivity in a study comprising nonobese subjects (198). In contrast, three independent studies could not demonstrate a clear association of this alanine substitution with obesity, fat distribution, or type 2 diabetes mellitus (199, 200), or with lipoatrophic diabetes (201). Finally, the pro12ala mutation was found associated with an increased BMI in two different Caucasian populations (202). These contradictions emphasize the difficulty to assess the role of a given mutation in the multifactorial and polygenic disorder of obesity. Another mutation, a proline-to-glutamine substitution at position 113 of PPARγ (given as position 115 in the original paper), has been found in 4 of 212 obese subjects *vs.* none in 237 subjets of normal weight. All four subjects with the mutant allele were markedly obese, with BMI values significantly higher as compared with the mean in the other obese subjects. In *in vitro* assays, this mutation inhibits the phosphorylation of Ser112, a target of MAP kinase, and increases PPARγ activity in an adipogenic test (126). Finally, a genome wide screen for type 2 diabetes mellitus conducted in Mexican-American affected sib pairs did not reveal any linkage to the D3S1263

TABLE 3. PPAR target genes with identified PPREs

Target genes	Gene function	References
Acyl-CoA synthase	Fatty acid activation	(223)
Acyl-CoA oxidase	Peroxisomal β-oxidation	(15, 23, 29, 401, 402)
Apolipoprotein A-I	Blood transport of fatty acid	(403)
Apolipoprotein A-II	Blood transport of fatty acid	(404)
Apolipoprotein C-III	Blood transport of fatty acid	(23, 405)
aP2 adipocyte lipid binding protein	Intracellular fatty acid binding	(183)
Bifunctional enzyme (enoyl-CoA hydratase/3-Hydroxy-acyl-CoA dehydrogenase)	Peroxisomal β-oxidation	(227, 406)
CPTI carnitine palmitoyl transferase I	Entry of fatty acyl into mitochondria	(233–235)
Cyp4A1/P450 IV family	Microsomal ω-oxidation	(255)
Cyp4A6/P450 IV family	Microsomal ω-oxidation	(23, 254)
Fatty acid transport protein	Fatty acid transport across cell membrane	(284)
Lipoprotein lipase	Fatty acid release from lipoprotein-bound triglycerides	(283)
Liver fatty acid binding protein	Intracellular fatty acid binding	(407)
Liver-specific type I sugar transporter	Sugar transport	(32, 408)
Malic enzyme	Fatty acid synthesis/NADPH production	(246)
Medium chain acyl-CoA dehydrogenase	Mitochondrial β-oxidation	(236)
Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase	Ketone body synthesis	(237)
Phosphoenolpyruvate carboxykinase	Glycerogenesis (adipose tissue)	(242)
Scavenger receptor CD36	Uptake of modified LDL in macrophage	(344)
Stearoyl-CoA desaturase 1	Desaturation of fatty acyl-CoA	(249)
Uncoupling protein 1 (brown adipocyte)	Nonshivering thermogenesis	(298)

Only genes for which a functional PPRE has been identified are listed, with the corresponding references. The function or pathway in which these target genes are involved is also indicated.

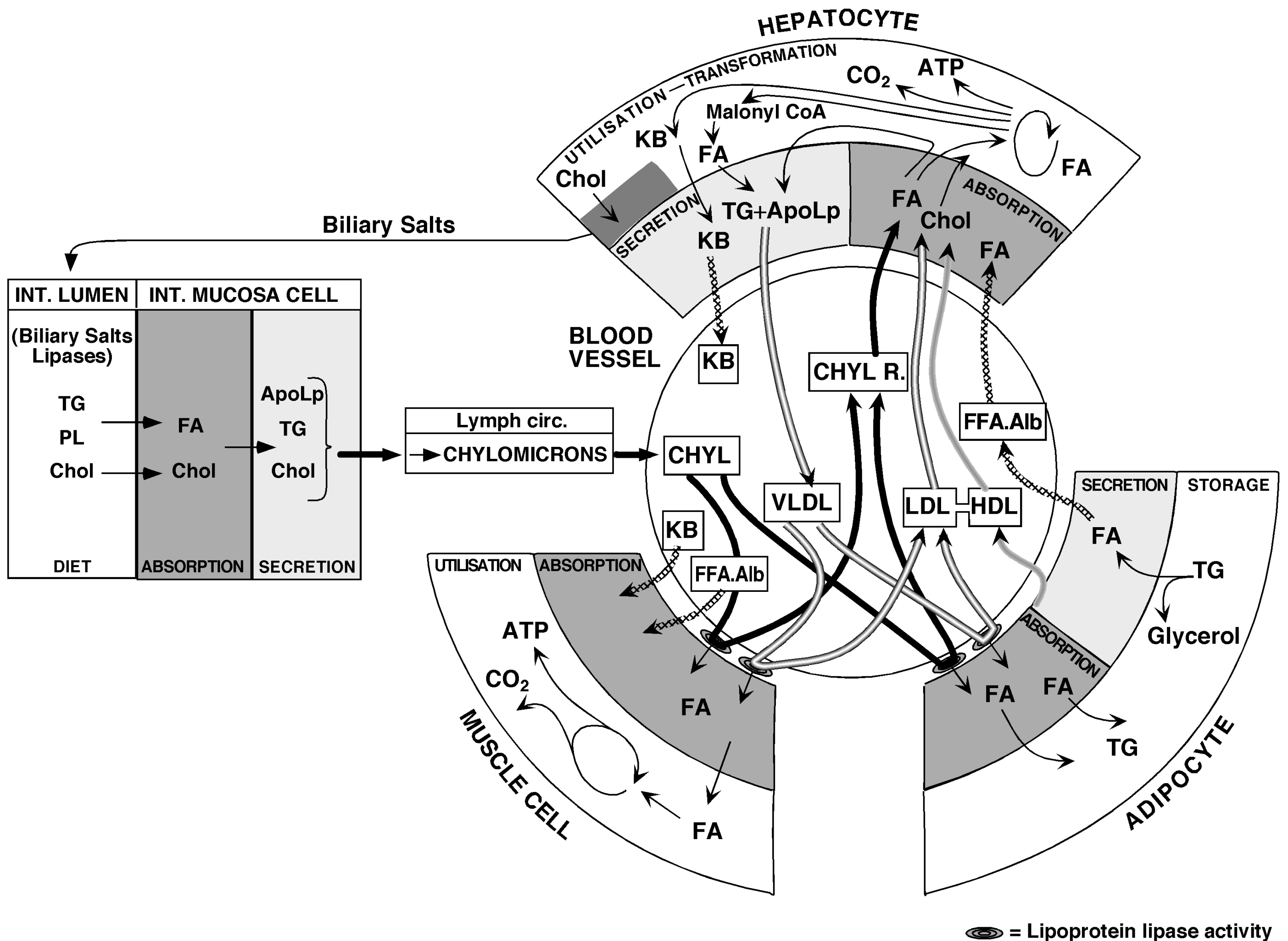

FIG. 4. Lipid transport and utilization from intestinal lumen to liver, adipose, and muscular tissues. A scheme illustrates the main features of the different pathways involved. The absorption and processing of the lipids (triglycerides, TG; cholesterol, Chol; phospholipids, PL; fatty acids, FA) in the intestinal mucosa generate the chylomicrons (CHYL), which circulate in the lymph and are delivered to the blood. FA uptake by muscle cells (energy substrate) and adipocytes (storage in form of TGs) after their release from TG by the extracellular lipoprotein lipase produces chylomicron remnants (CHYL R.), which are cleared from the blood by the hepatocytes. The fatty acid reesterified in the hepatocytes are secreted in form of TGs associated with apolipoproteins (ApoLp) in VLDL particles. LDL particles, which are depleted in FA by the action of lipoprotein lipase but enriched in cholesterol, as well as the HDL particles, which participate in the reverse transfer of cholesterol from peripheral cells to the liver, are taken up by hepatocytes. In energy-demanding situations, lipolysis in adipocytes triggers the release of FFA whose main part is transported in the blood by albumin (FFA.Alb), whereas the hepatocytes produce ketone bodies (KB) as fuel for peripheral tissues (muscle, brain, kidney). This figure is not comprehensive and is meant as an aid to illustrate the connected pathways controlled by PPARs.

marker, which is within 1.5 Mb from the PPARγ gene (203), whereas evidence for linkage at 3p24.2-p22, *i.e.*, in the vicinity of RARβ and PPARγ genes, was found in obese Pima indians (204). In conclusion, because obesity and diabetes are multifactorial and polygenic diseases, very large-scale studies and/or linkage analyses will be necessary to ascertain the impact of a given mutation with respect to these phenotypes.

B. PPAR target genes and functions in fatty acid metabolism

The first major insights into PPAR biology came from the demonstration of PPARα-mediated control of liver peroxisomal β-oxidation (15, 29) and of the role of PPARγ in adipogenesis (183). Thus, the search and identification of PPAR target genes (Table 3) have been mainly concentrated on hepatocytes and adipocytes, which both play a key role in systemic lipid metabolism, and indeed indicate that PPARs exert a general regulatory effect on lipid homeostasis. However, many other aspects of PPAR physiological roles, often linked to lipid-specific functions, are currently being unveiled and will be discussed in the following chapters. As an aid in placing PPAR-specific action in a broader physiological context, we propose a short and necessarily simplified summary of the pathways of interest.

Fat in mammals can be endogenously synthesized, using lipids, carbohydrates, or amino acids. However, the main source of fat in mammals is their direct absorption from the diet (Fig. 4). Upon absorption from the gut lumen by the intestinal villi cells, which requires triglyceride hydrolysis in the lumen and reesterification in epithelial cells, lipids are

delivered to the general circulation via the lymph as chylomicrons, which are mainly composed of triglycerides, cholesterol, and lipoproteins. An extracellular enzyme, the lipoprotein lipase, located at the surface of vascular endothelial cells, hydrolyzes the triglyceride component of the chylomicrons, thereby delivering fatty acids to target cells. Chylomicron remnants, which are enriched in cholesterol, then enter hepatocytes by endocytosis or after hydrolysis by the hepatic lipase. Other fatty acids present in the bloodstream are nonesterified and mainly carried by serum albumin. For most of them, they originate from the adipose tissue where they are released upon lipolytic stimulation, such as that provoked by catecholamines or glucagon. The fate of fatty acids in the liver is dependent on the energy status of the organism. The reesterification pathway, leading to triglyceride release into the systemic circulation in the form of very low density lipoproteins (VLDL), is quantitatively predominant in any case but particularly favored when energy fuels (carbohydrate and fatty acids) are abundant. The fatty acid content of VLDL is mainly taken up by adipose tissue for storage. Conversely, when plasma fatty acid levels are high and carbohydrates low, the oxidative pathway leads to the production and secretion of ketone bodies that serve as lipid-derived fuel for brain, muscle, kidney, and other peripheral tissues during exercise, starvation, or energy metabolism-related diseases (*e.g.*, in the case of the relative or absolute insulin deficiency in diabetes). Thus, the liver is able to regulate the levels of the three forms of circulating "fat," *i.e.*, nonesterified fatty acids, triglycerides, and ketone bodies, by modulating the relative rates of fatty acid uptake, esterification into triglycerides, and oxidation, respectively. The adipose tissue is another site of important regulatory pathways and hormonal cross-talk that actively contributes to lipid metabolism homeostasis. Several PPAR target genes that are involved in these pathways, from the intestinal villi to the adipocyte, have been identified and together with the preferential expression of PPARα in the liver and PPARγ in the adipose tissue, they provide a firm basis for understanding the physiological roles of PPAR with respect to lipid metabolism and energy homeostasis, as we will now examine.

With reference to the general display of Fig. 4, PPAR functions and target genes in lipid metabolism and energy homeostasis will be presented in the four following sections: 1) PPARs in the digestive tract; 2) PPAR, circulating lipoproteins, and cholesterol metabolism; 3) the pleiotropic role of PPARα in the liver; and 4) PPARγ and adipogenesis. Since relatively little is known concerning specific functions of PPAR in muscle, no distinct chapter is devoted to the subject.

1. PPARs in the digestive tract. Triglycerides and phospholipids from the diet are mainly absorbed in the duodenum and jejunum, while cholesterol is mainly absorbed in the ileum. In these intestinal regions, the high PPARα and PPARβ expression correlates with the expression of the enterocytic fatty acid binding protein (FABPs) genes, the I-FABP and L-FABP, and of the cellular retinol binding protein genes (205, 206). A strong positive regulation of the L-FABP gene occurs upon dietary intake of long-chain fatty acids or direct ileal infusion of linoleic acid or α-bromopalmitate, which are PPARα and PPARβ ligands, whereas I-FABP was unaffected (167, 206, 207). A clofibrate-enriched diet also induces L-FABP gene expression (208) and further suggests a relationship between PPAR and FABP expression in the gut. Another putative target gene is that of the fatty acid translocase (FAT) which has been proposed to facilitate the transport of long-chain fatty acids into the enterocyte. It is related to the docking receptor CD36, and its expression pattern and regulation of expression closely resemble that of FABP in the small intestine (209). Other genes involved in the transformation of long-chain fatty acids into triglycerides and their incorporation into chylomicrons or VLDL particles, such as those coding for the acyl-CoA synthase (ACS) or for the apolipoproteins, are also PPAR target genes, as will be discussed later. Specific functions for PPAR in the colon, in which the three isotypes are expressed, have yet to be defined. Using an antibody directed against the LBD of PPARα but which cross-reacts with PPARγ, Mansen *et al.* (184) showed an increasing gradient of PPAR expression from the crypt to the top of the colon villi, which suggests a role of PPAR in fatty acid absorption. The precise localization of the PPARγ isotype, cryptic or at the top of the villi, remains under debate and raises questions about the link between PPARγ, cell proliferation, and cell maturation in the intestinal mucosa (see also *Section III.F*) (185, 186).

2. PPAR, circulating lipoproteins, and cholesterol metabolism. Cholesterol is an essential component of cell membranes and is the molecule from which steroids are synthesized and which serves as precursor for bile acid synthesis. The main source of cholesterol is the diet. If this supply is insufficient, then cholesterol synthesis is induced mainly in liver cells but also in many other cells. Two transcription factors are currently known to have a strong impact on intracellular cholesterol metabolism: SREBP (1) and LXRα (3). Little is known about the putative role of the different PPAR isotypes with respect to regulation of cholesterol, except that cellular cholesterol levels in preadipocyte influence PPARγ expression (see Ref. 274). This effect is mediated by adipocyte determination and differentiation factor 1 (ADD1)/SREBP1, which is preferentially involved in fatty acid synthesis, whereas SREBP2 plays a role in intracellular cholesterol metabolism. Such functional interactions between transcription factors suggest interconnected regulations of cholesterol and fatty acid metabolism.

In addition to intracellular cholesterol metabolism, the regulation of circulating cholesterol levels has a high physiopathological relevance since, qualitatively and quantitatively, it is a risk factor for atherosclerosis and its associated diseases. The cholesterol-enriched low density lipoprotein (LDL) particles are formed by release of the triglyceride content of VLDL via the action of lipoprotein lipase (LPL) (see Fig. 4). The released fatty acids are either stored in the adipose tissue or oxidized to generate ATP in different tissues, especially muscle. High density lipoprotein (HDL), in contrast to LDL, is considered as a "good" cholesterol-containing lipoprotein particle as it has a protective effect on atherosclerosis development. Indeed, it is instrumental in removing excess cholesterol from extrahepatic cells and in transporting it to the liver and steroidogenic organs, where

it is taken up via the scavenger receptor BI (210). The role of PPARs in this general picture is reflected by the therapeutic benefits of fibrates, which are the first efficient lipid-lowering drugs to be used. Fibrate treatment both enhances catabolism of triglyceride-rich particles and reduces VLDL production. Furthermore, it stimulates HDL apolipoprotein expression (211). One other important mechanism of the fibrate lipid-lowering effect is believed to be an increased LPL activity through PPAR-mediated activation of LPL gene expression. LDL and HDL blood levels also depend in part on the synthesis, mainly by the liver, of the apolipoproteins required for their assembly. Several of these apolipoproteins are regulated by fibrates via PPARs. Fibrates down-regulate the production of apoCIII (117), an atherogenic component of apoB-containing lipoproteins, which inhibits LPL activity and impairs the uptake by the liver of triglyceride-rich lipoproteins. Direct support for these apoCIII effects is provided by transgenic animal studies showing a correlation between liver apoCIII expression and plasma triglyceride levels (212). Consequently, down-regulation of hepatic apoCIII production by PPARα appears to be beneficial since lipolysis of VLDL particles is increased and the resulting LDL is efficiently removed from the plasma. In humans, apolipoprotein AI and AII, which are the major HDL apolipoproteins, are up-regulated by PPAR through transcriptional control, while ApoAI expression is down-regulated in rodents. Consistently, a PPRE was identified in the human ApoAI promoter, whereas there is no PPRE in the rat promoter. In the latter, the fibrate-dependent repression is mediated by the nuclear receptor Rev-erbα, which binds to a negative element and whose gene is a fibrate target gene (213). The PPRE of the human ApoAI and ApoAII promoters can also be occupied by RXR homodimers or by other transcription factors such as ARP1, HNF4, EAR2, or EAR3, resulting in a complex and, so far, unclear pattern of regulation (214–216). Finally, apolipoprotein B secretion as well as VLDL production are inhibited by peroxisome proliferators by mechanisms not yet characterized (217). Importantly, all of the above-mentioned apolipoprotein genes (i.e., apoCIII, apoB, apoAI, and apoAII) and the apoAIV gene were previously shown to be regulated by HNF4 (Ref. 218 and references therein). This raises the general question of interference between PPAR and HNF4 signaling, more specifically as to whether other known HNF4 target genes, such as the human coagulation factors VII, IX, and X (Ref. 218 and references therein) or the liver-enriched transcription factor HNF1 (219), are also regulated by PPAR.

One difficulty in interpreting these observations comes from the fact that the various fibrates, although primarily activating PPARα, may also have effects on the two other isotypes, most likely resulting in a broad spectrum of related but distinct activities. Finding better isotype-specific ligands might increase control over biological effects but, paradoxically, by being more selective, these ligands may also reduce the efficiency in treating complex disorders such as dyslipidemia. In this context, it will be of major interest to understand the mechanism by which a PPARβ-specific ligand can increase the level of HDL, as reported recently (220).

3. Pleiotropic roles of PPARα in the liver.

Regardless of the fate of fatty acids in the liver, two first steps – fatty acid transport across the cell membrane and activation into an acyl-CoA – are required for further processing of the fatty acids. These two steps are facilitated through the induction of a fatty acid transporter protein (FATP) and FAT by ligand-activated PPARα (221, 222) as well as by the up-regulation at the transcriptional level of the long-chain fatty acid ACS gene (223). Formation of fatty acyl-CoA by ACS precedes either their incorporation into triglycerides (the anabolic pathway) or their oxidation (the catabolic pathway) by two major pathways: peroxisomal β-oxidation and mitochondrial β-oxidation. For each of these pathways, the expression of some key enzymes is up-regulated by PPARα.

a. PPARα and peroxisomal β-oxidation: Peroxisome proliferation, which can be triggered in rodents but not in humans, corresponds to an increase in the volume density of peroxisomes and of the peroxisomal fatty acid β-oxidation activity. This activity is inducible by signals such as exposure to cold, high-fat diet, and thyroid hormone, but also by a wide variety of compounds collectively called peroxisome proliferators that includes certain hypolipidemic drugs (224). As mentioned above, the name of these receptors is derived from the first discovered PPARα activators, which belonged to this class of compounds (5). The fact that PPARα KO mice cease to exhibit peroxisome proliferation upon exposure to the classic peroxisome proliferators, clofibrate and Wy-14,643, demonstrate that PPARα is indeed the main mediator of the pleiotropic actions of this class of compounds (225). Accordingly, the first PPAR target genes that have been characterized encode peroxisomal enzymes, more specifically the enzymes of the β-oxidation pathway. The genes encoding acyl-CoA oxidase (ACO), which is the rate-limiting enzyme in the pathway, enoyl-CoA hydratase/dehydrogenase multifunctional enzyme (HD), and keto-acyl-CoA thiolase are direct targets of PPARα (15, 29, 226, 227). In contrast, neither the catalase gene nor the urate oxidase gene, which control the disposal of the H_2O_2 produced by fatty acid oxidation, appears to be directly regulated by PPARα. Peroxisomes also contain enzymes participating in cholesterol and dolichol synthesis, and in the oxidative degradation of polyamines, purines, and D-amino acids. A direct role of PPAR in these pathways has not been reported.

What is the physiological role of PPARα as a mediator of peroxisomal proliferation? With respect to energy homeostasis, peroxisomal β-oxidation is not directly coupled to an electron transport chain and oxidative phosphorylation. Therefore, the energy released in the first oxidation step (H_2O_2 production) is lost as heat, and the energy released in the second step is conserved in the form of the high-energy level electrons of NADH (228). Peroxisomal β-oxidation appears to be mainly a chain-shortening mechanism of the very long-chain fatty acids (>C20), which predominantly come from the diet and are prevented from entering mitochondria. After a few rounds of peroxisomal β-oxidation, which removes two carbons at each round in the form of an acetyl-CoA molecule, the shortened chain can then be further de-

graded to completion in the mitochondrion. The acetyl units (2-carbons), which are generated by the peroxisomal pathway, can be converted to acetylcarnitine, acetate, and acetoacetyl-CoA. Alternatively, they can be used by the fatty acid chain elongation system or serve other biosynthetic purposes in the cytosol (*e.g.*, sterol synthesis) illustrating the substantial role of peroxisomes in fatty acid recycling (228, 229). Moreover, an isotopomer analysis in HepG2 cells demonstrated that peroxisomal fatty acid chain shortening induced by a PPAR ligand (troglitazone) might also be important for the shortening of saturated fatty acids and might contribute to membrane lipid synthesis (230). Finally, peroxisomal β-oxidation also oxidizes other substrates, such as some eicosanoids and xenobiotics, which are then excreted in the urine as metabolites (228). Thus, PPARα, by stimulating peroxisomal β-oxidation, on the one hand, helps in furnishing fatty acid substrates that can enter the mitochondrion or be used in membrane synthesis and, on the other hand, contributes to the detoxification of endogenous and exogenous active molecules, some of which may be PPAR ligands.

b. PPARα and mitochondrial β-oxidation: Mitochondrial β-oxidation greatly contributes to energy production via oxidative phosphorylation generating ATP. The role of PPARα in energy homeostasis is linked to the extent with which PPARα regulates this pathway. As far as energy conservation is concerned, mitochondrial β-oxidation is approximately twice as efficient as peroxisomal β-oxidation. The first limiting step in mitochondrial β-oxidation is the entry flux of fatty acids into the mitochondria, which is controlled by a carnitine-dependent facilitated transport system. This control is not only quantitative but also qualitative since it excludes the very-long-chain fatty acids ($C > 20$). One of its critical components, the carnitine palmitoyl transferase I (CPT I), catalyzes the formation of fatty acyl carnitine for translocation across the inner mitochondrial membrane. This enzyme is strongly induced by peroxisome proliferators and fatty acids (231, 232), and a functional PPRE has been characterized in the promoter sequence of the muscle-type CPT I gene (233–235). PPARα further regulates the mitochondrial β-oxidative spiral by modulating the expression of the medium-chain acyl-CoA dehydrogenase (MCAD) gene (236).

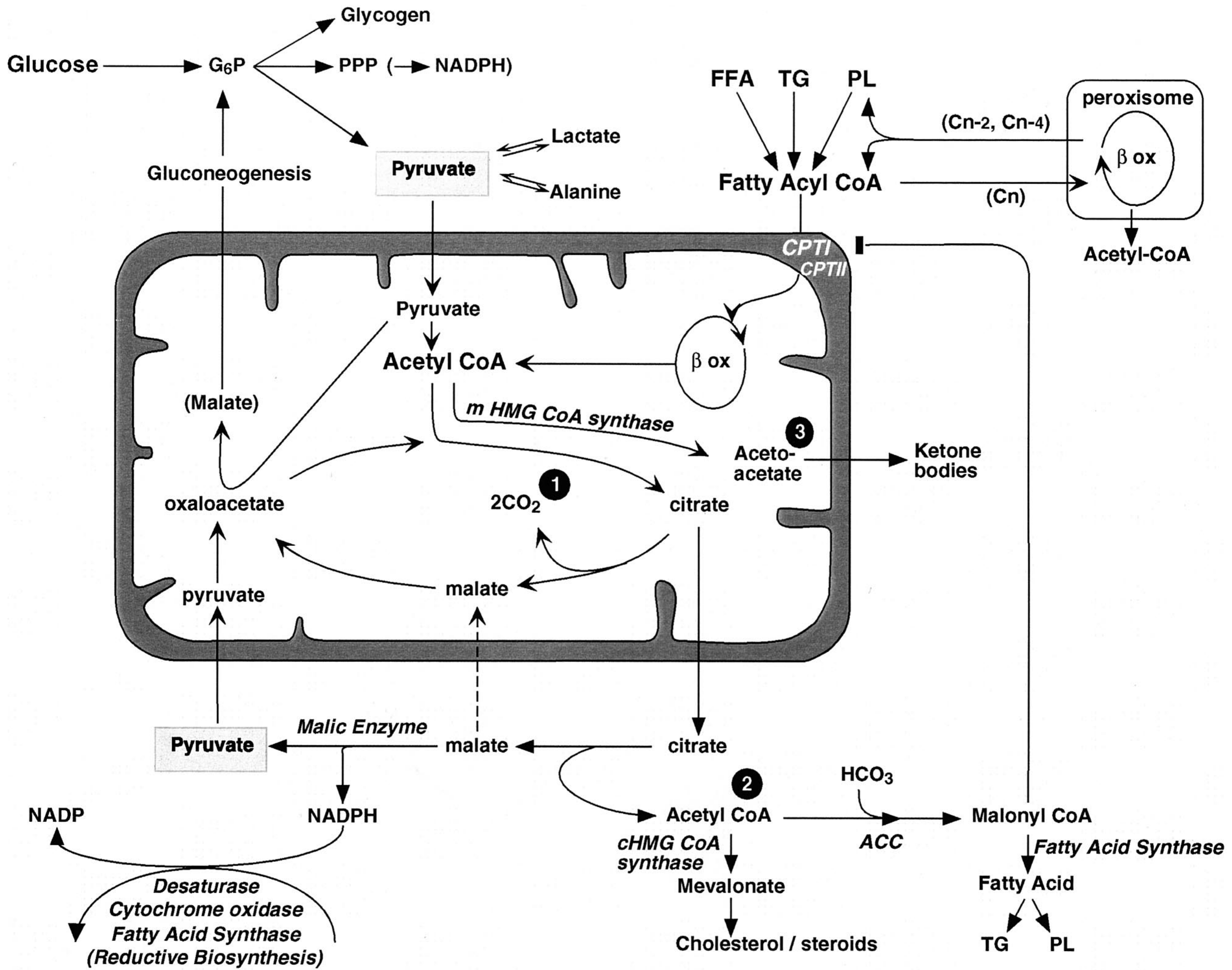

FIG. 5. Fatty acids and energy metabolism. The main pathways that lead to the utilization of fatty acids as energetic substrates and are targets of PPARα action are depicted. The three "devenirs" of acetyl-CoA units are marked by circled 1, 2, and 3. Attention was particularly given to enzymes cited in the text. ACC, Acetyl CoA carboxylase; G6P, glucose 6 phosphate; PPP, pentose phosphate pathway; TG, triglycerides; PL, phospholipids; β ox, β oxidation; CPT, carnitine palmitoyl transferase.

The acetyl-CoA unit produced at each cycle of fatty acid β-oxidation in mitochondria has three possible fates (Fig. 5): 1) acetyl-CoA condenses with oxaloacetate, normally provided by the glycolytic pathway via pyruvate, to form citrate, which can enter the citric acid cycle for complete oxidation to CO_2 and ATP generation; 2) alternatively, the citrate resulting from the condensation of acetyl-CoA with oxaloacetate is exported into the cytosol for the synthesis of fatty acids or other purposes; 3) if oxaloacetate is low or unavailable because of its use in gluconeogenesis, as seen during fasting or in diabetes, a major portion of the acetyl-CoA is converted to ketone bodies, mainly acetoacetate and 3-hydroxybutyrate. These molecules serve as important energetic substrates for extrahepatic tissues such as skeletal muscle, heart, kidney cortex, and the brain for which it is the only non-glucose-derived source of energy. The mitochondrial hydroxymethylglutaryl-CoA synthase (mHMG-CoAS) is the main enzyme involved in ketone body formation and is directly controlled by PPARα (237). Surprisingly, the mHMG-CoAS protein can interact with PPARα *in vitro* via a LXXLL motif also used by coactivators for interaction with the receptors (see *Section II.E.2*). *In vivo*, in the presence of PPARα, mHMG-CoAS is translocated into the nucleus and potentiates PPARα-dependent transcription activation of the mHMG-CoAS gene specifically via the HMG-CoAS PPRE. These interesting findings reveal a novel mechanism whereby the product of a PPARα target gene, which functions as a ketogenic enzyme in mitochondria, also specifically autoregulate its own nuclear transcription by modulating the activity of PPARα (238).

PPARα KO mice are very informative about the respective importance of PPAR in peroxisomal *vs.* mitochondrial β-oxidation. A first characterization of these animals showed that both pathways are no more responsive to Wy-14,643 stimulation (225). Further analyses revealed that the basal expression of seven mitochondrial enzymes in the liver, including very-long-chain acyl-CoA dehydrogenase, long chain acyl-CoA dehydrogenase, long chain acyl-CoA synthetase, and short chain-specific 3-ketoacyl-CoA thiolase, are lower in PPARα KO mice *vs.* wild-type animals (239). This latter study underlines the importance of PPARα for the constitutive level of mitochondrial β-oxidation. In contrast, the basal expression level of peroxisomal genes is not affected by functional neutralization of the PPARα gene. However, and as a general note of caution, one must be aware that it is difficult to rule out that the adult "metabolic" phenotypes observed actually reflect a possible effect on liver development of the deletion of the PPARα gene.

c. PPARα and adaptation to fasting and stress: Fasting and stress represent typical situations in which coordination in the liver of PPARα expression and its activation results in an enhanced breakdown of fatty acid into energy-rich units. In such situations, lipolytic stimulation in the adipose tissue increases the plasma levels of nonesterified fatty acids, whose rapid uptake by the liver increases the intracellular concentration of these PPARα activators. Concurrently, PPARα expression is directly stimulated by elevated circulating glucocorticoid levels (173, 174), while levels of insulin, which counteracts many effects of glucocorticoids, are decreased (172). Fasting is also associated with a rapid depletion of the glycogen stores and an increased rate of gluconeogenesis. PPARα null mice have low glycogen stores and, upon fasting, they exhibit a severe hypoglycemia and hypothermia (240). These manifestations are accompanied by an enhanced lipid accumulation in the liver and no increase in ketone body production, suggesting a dramatic impairment of fatty acid oxidation (240, 241). Another link between PPARα and gluconeogenesis is revealed by treatment of wild-type and KO mice with etomoxir, an agent that blocks CPT I activity. This treatment provokes a lethal hypoglycemia in the PPARα KO mice. In contrast, wild-type animals tolerate etomoxir treatment and respond by a strong up-regulation of known PPAR target genes (ACO, CYP4A1, CYP4A3, and MCAD). The hypothesis, consistent with the above-mentioned observations, is that PPARα KO mice suffer from both a depletion of glycogen stores and diminished gluconeogenesis, due to a low acetyl-CoA/long-chain acyl-CoA ratio that inhibits pyruvate carboxylase, a rate-limiting enzyme in hepatic gluconeogenesis. In concert, there is a marked triglyceride accumulation in liver and heart of the dead mice. Another key regulatory enzyme of gluconeogenesis in the liver is PEPCK. The promoter of its gene contains a functional PPRE (242) and therefore might be stimulated by PPARα in liver. However, this response element is located in the distal enhancer region of the promoter, which has been shown to be involved mainly in adipocyte-regulated PEPCK expression, and there is no alteration of the PEPCK gene expression in the liver in PPARα KO mice (240).

Intriguingly, survival of PPARα KO mice treated with etomoxir presents a strong sexual dimorphism as 75% of PPARα KO females or estradiol-pretreated males survive vs. no survival of PPARα KO males. The pathway and mechanism of this estrogen-dependent rescue are not yet understood (243). A remarkable sexual dimorphism of the PPARα KO mice was also observed when they were identified as a model of monogenic, late-onset obesity (244). Females develop a more pronounced obesity than males which, in turn, present a marked steatosis in liver associated with the delayed occurrence of obesity. These observations link PPARα with a sexual dimorphic control of circulating lipids, fat turnover, and obesity. Another sexual dimorphism that affects the activity of PPARγ is the estrogen-induced peroxisome proliferation in the duck uropygial gland, the function of which is to produce 3-hydroxy fatty acid esters that serve as female pheromones during the mating season (245). Expression of PPARγ1 is high in this organ, and estrogen induces the formation of a PGD2 metabolite similar to Δ^{12}-PGJ2, able to activate the receptor. These findings raise the possibility that in this tissue, PPARγ might be responsible for peroxisome proliferation. While the extent of sexual dimorphism, particularly that of the PPARα KO mice, might depend on the genetic background or other poorly controlled parameters, these observations certainly invite further investigations of the cross-talk or interference between ER and PPAR signaling.

d. PPARα and liver fatty acid synthesis: The idea of PPARα being involved in fatty acid synthesis stems from the demonstration that the lipogenic malic enzyme gene is up-reg-

ulated by peroxisome proliferators via PPARα through a well-characterized PPRE (31, 246). Moreover, basal expression of this gene in the liver is lowered in PPARα KO mice (239). The reaction catalyzed by malic enzyme consists of the oxidative decarboxylation of cytosolic malate, which generates pyruvate and leads to the formation of NADPH, required for lipid synthesis (Fig. 5). However, the role of PPARα with respect to fatty acid synthesis in the liver appears complex since other important lipogenic genes are down-regulated by polyunsaturated fatty acids (PUFAs) and insensitive to other PPARα activators (247, 248). This raises two questions. 1) Does the up-regulation of the malic enzyme gene truly indicate a role of PPARα in fatty acid synthesis?; and 2) Are the regulatory events triggered by PUFAs mediated by PPAR? In an attempt to answer the first question, three hypotheses can be discussed. The activation of both catabolism and anabolism of the same molecules, *e.g.*, fatty acids, would correspond to a futile cycle wasting energy and generating heat. This cycle cannot be much used since malonyl-CoA, which is the first and committed product in fatty acid synthesis, is an inhibitor of the CPT I enzyme, thereby inhibiting mitochondrial fatty acid oxidation (Fig. 5). Thus, from this network of regulation, it appears unlikely that the PPARα-mediated activation of the malic enzyme gene in the liver is dedicated to lipid synthesis. Alternatively, NADPH is not only required for fatty acid synthesis but is also involved in many reductive biosynthetic pathways. A first example is that of the liver cytosolic enzyme stearoyl-CoA desaturase 1 (SCD-1), which catalyzes Δ^9-*cis* desaturation of saturated fatty acyl-CoA substrates and requires NADPH as the second substrate for oxidation. This desaturation increases the ratio of oleic acid *vs.* stearic acid in membrane phospholipids and thus modifies the membrane fluidity. It has also been associated with a facilitation of fatty acid incorporation into VLDL particles. This gene is itself up-regulated by PPARα and peroxisome proliferators (249). Other examples of NADPH-dependent reactions are those catalyzed by mixed-function oxygenases (cytochrome oxidases). Whereas much of the NADPH required for these pathways is provided through the pentose phosphate pathway, the transfer of reducing equivalents from the mitochondria to the cytosol, by means of the malic enzyme shuttle, was previously suggested as being important for monooxygenase functions (250). This is consistent with the role of PPARα and fibrates in inducing some of these monooxygenase activities (see below). Finally, one might consider that the activation of the malic enzyme gene increases the amount of pyruvate, which is one major metabolic junction linking glucose, amino acids, and lipid metabolism (Fig. 5).

The second question concerns the mechanisms of action of PUFAs. In most cases, up-regulation of PUFA target genes has been convincingly demonstrated as occurring through PPARα and is reproduced when using characterized PPARα ligands other than PUFAs. In contrast, the PUFA-dependent down-regulation of the Spot14 gene (whose function is unknown) as well as that of the fatty acid synthase (FAS) gene are not observed when using these other PPARα ligands, suggesting the existence of an alternative mechanism of action for PUFAs (248). The SCD-1 gene mentioned above also is up-regulated by fibrates but down-regulated by PUFAs (249). The malic enzyme gene itself is down-regulated by high-fat diet, although the molecular mechanism has not yet been elucidated. The existence of distinct pathways for PUFA/PPARα-dependent up-regulation and PUFA-induced down-regulation of target genes is further substantiated in PPARα KO mice where PUFAs cannot up-regulate the PPARα target gene ACO as expected, whereas they continue to down-regulate FAS and Spot14 gene expression, demonstrating that this down-regulation is PPARα independent (251). The involvement of the nuclear receptor HNF-4α in this PUFA-induced down-regulation has been proposed and is supported by the fact that competition occurs between PPARα and HNF-4 for binding to the same response element, as previously mentioned. Moreover, fatty acyl-CoA ligands can modulate the activity of HNF-4α by either activating or suppressing its action as transcription factor, depending on the chain length and degree of saturation (252). Thus, it is possible that HNF-4α is the factor mediating the PUFA-dependent down-regulation of lipogenic enzymes.

e. PPARα and microsomal ω-oxidation: The cytochrome monooxygenase system plays a central role in the oxidation of a wide variety of endogenous as well as exogenous compounds. The CYP4A enzymes participate in this system as a distinct group of the cytochrome P450 superfamily. They catalyze the ω-hydroxylation of fatty acids and eicosanoids and are induced by fibrates and other peroxisome proliferators in liver and kidney. ω-Hydroxylation is, for example, the first step in the neutralization of LTB4, a PPARα ligand, which is then completely degraded through β-oxidation in the peroxisomes (253). At least two of the CYP4A genes, CYP4A1 and CYP4A6, contain a functional PPRE in their promoter sequence and indeed respond *in vivo* and in cell culture to PPARα activators (23, 254, 255). By comparing PPARα-deficient and wild-type mice, it was shown that insulin-dependent diabetes and starvation result in a strong induction of the hepatic CYP4A genes and other lipid-metabolizing enzymes, such as ACO, through activation of PPARα (256). Three main conclusions can be drawn from all these observations: 1) PPARα can provide a negative feedback on the intracellular levels of an endogenous ligand (65); 2) a pathophysiological state can induce cellular changes that lead to the activation of PPARα (256); and 3) PPARα may have an important role in the detoxification of some xenobiotics. A better assessment of these PPAR detoxification functions is of great importance in the perspective of pharmacological and therapeutic applications.

4. PPARγ and adipogenesis.

a. PPAR and the adipocyte differentiation program: The first striking specific characteristic of PPARγ, when it was discovered, was its high expression levels in adipose tissue (15). A direct role of PPARγ in adipogenesis was then suggested by the fact that whereas preadipocyte cell lines, including 3T3-L1 and 3T3 F442A cells, express only trace amounts of PPARγ, the appearance of PPARγ during adipocyte differentiation precedes that of several markers of late differentiation, such as aP2, PEPCK, and CAAT/enhancer binding protein (C/EBPα) (183, 257). Moreover, PPAR activators such as Wy-14,643, ETYA, and TZDs, were able to promote

the conversion of preadipocytes into adipocytes (62, 258–260). Additional strong evidence for the adipogenic role of PPARγ came from its forced expression in NIH-3T3 fibroblasts which, in the presence of various activators (ETYA, clofibrate, LY 171883, and linolenic acid), underwent adipose differentiation and accumulated lipids (257). A similar adipogenic effect of PPARγ was observed in several fibroblastic cell lines (NIH-3T3, BALB/c-3T3, Swiss-3T3), whereas high PPARγ expression is found in lipid-laden lung fibroblasts (261).

The C/EBP family of transcription factors is also involved in the control of the adipocyte differentiation program (262). The expression of the three C/EBP isotypes, α, β, and δ, follows a specific pattern along the adipose differentiation process. A first transient increase of C/EBPβ and δ expression is followed by the onset of C/EBPα expression during the late phase. In this cascade, the appearance of PPARγ seems to intercalate between the C/EBPβ/δ and C/EBPα waves (183, 263). Indeed, C/EBPβ expression together with dexamethasone treatment lead to induced PPARγ expression and, consistently, C/EBP binding sites have been found in the PPARγ2 promoter sequence (264). At the time point of strong PPARγ expression in differentiating cells, addition of a PPARγ activator triggers lipid accumulation (263) as well as C/EBPα expression, both PPARγ and C/EBPα being required for establishment of insulin-sensitive glucose transport (265). Further evidence for the involvement of C/EBPβ in early steps of this cascade comes from a recent study of the TLS-CHOP oncoprotein, which is a fusion translocation liposarcoma-C/EBP homologous protein, found specifically in a malignant tumor of adipose tissue. TLS-CHOP forms heterodimers that cannot bind DNA with C/EBPβ. Therefore, C/EBPβ function is inhibited and adipose differentiation is blocked, but overexpression of PPARγ2 can overcome this blockage in tumor cells (266). In agreement with the role of C/EBPα as end point in the adipogenic program, C/EBPα KO mice have severe reduction of brown fat and white fat mass (267). However, a single linear cascade of events is not sufficient to explain adipogenesis since mice that are null allele mutants for both C/EBPβ and C/EBPδ exhibit an apparent normal expression of C/EBPα and PPARγ but have impaired adipogenesis (268). Inhibition of all three C/EBPs by overexpression of a dominant-negative protein A-ZIP/F-1 also results in normal PPARγ expression but no fat development (269). Conversely, optimal differentiation requires a combination of factors as seen, for example, during the adipocyte conversion of 3T3 fibroblasts in which C/EBPβ, C/EBPδ, and dexamethasone are necessary to induce PPARγ expression. A sustained expression of C/EBPβ seems also to be important for full PPARγ activity (270).

Transcription factors involved in adipogenesis also comprise the sterol regulatory element binding proteins (SREBPs). SREBP-1a and -1c are not absolutely required for fatty acid synthesis and adipogenesis but rather act as auxiliary regulators (1). SREBP-1c, first identified as an adipocyte differentiation and determination factor called ADD1 (271), is induced early in adipose differentiation of mouse 3T3-L1 preadipocytes. Since conditioned medium from cells transfected with ADD1/SREBP contains an unidentified activator(s) for PPARγ (272), terminal adipocyte differentiation may result from an SREBP-induced production of this ligand, possibly a lipid. In contrast, overexpression of the nuclear form of SREBP1c in the adipose tissue of transgenic mice lowers PPARγ expression as well as that of other markers of adipocyte differentiation, generating a model of fat-deprived mice (273). This apparent paradox might reflect the importance of the timely schedule of SREBP1c gene activation in the adipocyte differentiation program. The other SREBP isotype, SREBP-2, is essential in regulating cholesterol metabolism. A response element to which SREBP binds has been characterized in the PPARγ3 promoter, and cholesterol depletion stimulated this promoter (274). This induction of PPARγ expression by cholesterol depletion may explain why agents reducing circulating cholesterol, such as the HMG-CoA reductase inhibitors, also lower triglyceride levels.

Growth factors and insulin also regulate adipogenesis; PDGF, EGF, and fibroblast growth factor (FGF) inhibit adipocyte conversion and, as already mentioned (Sections II.D.1 and *II.E.1*), this effect correlates with a phosphorylation of serine 82 and 112 in the N-terminal domain of PPARγ1 and PPARγ2, respectively, and with an inhibition of PPARγ transcriptional activity (97, 98). In contrast, insulin and insulin-like growth factor induce adipocyte differentiation and enhance transcriptional activity of PPARγ via a mechanism that needs to be elucidated. Werman *et al.* (124) speculate about the respective roles of the ligand-independent and ligand-dependent PPARγ domains (AF-1 *vs.* AF-2) under varying physiological and metabolic conditions to which adipocytes or cells expressing PPARγ are exposed. In the presence of high amounts of PPARγ ligand, adipocyte differentiation would be favored by the PPARγ-induced growth arrest and stimulation of specific adipocyte genes via the ligand-dependent AF-2 domain. When ligand is rare, growth factor-controlled PPARγ activity would regulate genes needed for basal adipocyte homeostasis via the ligand-independent AF-1 domain of the receptor.

In addition to PPARγ, PPARβ is also expressed at significant but lower levels in adipocytes. In ob1771 cells, its expression is activated shortly before the cells reach confluence and start to undergo phenotypic changes linked to differentiation. PPARβ was thus proposed as being an early initiator of the differentiation program (9). To compare the adipogenic potential of the three PPAR isotypes, their forced expression in fibroblasts followed by exposure to their respective ligands was performed. PPARγ has the best adipogenic impact and was the only isotype to be able to cooperate with C/EBPα. PPARα also was able to trigger a certain level of adipogenesis while, in this assay, PPARβ on its own was inefficient (275). However, forced expression and activation of PPARβ raise PPARγ expression, which, together with the addition of PPARγ ligand, leads to adipocyte differentiation (276).

During development, adipocytes originate from mesodermal pluripotent cells. Depending on the developmental stimuli, these progenitor cells differentiate into either myotubes, chondrocytes, or adipocytes. *In vitro* conditions for differentiation of embryonic stem cells into adipocytes are close to those for myotube differentiation, and a mixed culture of adipocytes and myocytes is obtained (277). Although in a single given cell these differentiation pathways are mutually

exclusive, some degree of plasticity remains. For example, the forced and simultaneous expression of PPARγ and C/EBPα in G8 myoblastic cells and treatment with PPAR activators inhibits myotube formation, represses the expression of several muscle-specific markers and triggers differentiation into adipocytes (278). This transdifferentiation also occurs in nontransfected C2C12 myoblasts exposed to the potent PPARγ agonist rosiglitazone 2 days before the culture reaches confluence (279). It is tempting to correlate this myotube transdifferentiation obtained in culture with the adipose tissue formation that occurs within muscle in certain pathologies such as in obesity (280). Finally, bone marrow stromal cells can differentiate *in vitro* into adipocytes when treated by TZD (281), which correlates with the inappropriate adipogenesis that might occur in bone marrow of dogs treated with TZD (282).

b. PPARγ target genes in adipose tissue: The phenotypical conversion of fibroblasts or stem cells into lipid-accumulating cells is accompanied by the induction of several specific adipose markers. A direct role of PPARγ in the up-regulation of many of the corresponding genes has been described. They encode enzymes involved in fatty acid release such as LPL (283), which is secreted by adipocytes and triggers the release of fatty acids from lipoprotein-bound triglycerides in the extracellular space. As in hepatocytes or in enterocytes, the incorporation of long-chain fatty acids into adipocytes might be facilitated by putative transport proteins, FAT and FATP, which are up-regulated by PPARγ and -α activators (222, 284). The adipocyte fatty acid-binding protein aP2 gene, as well as the acyl-CoA synthase gene, contains a functional PPRE within its promoter sequence and can be stimulated in differentiated adipocytes (183, 223). Fatty acid and triglyceride syntheses are also promoted by PPARγ-mediated activation of the malic enzyme gene (31, 246), which is clearly lipogenic in this context, and that of the PEPCK gene (242, 285) involved in the production of glycerol for storage of the fatty acids in the form of triglycerides. Finally, Wu et al. (286) demonstrated that the expression of the gene encoding the insulin-dependent glucose transporter GLUT4, which plays an important role in maintaining glucose homeostasis, is up-regulated by PPARγ and TZD. The c-Cbl-associated protein (CAP) also belongs to the insulin-signaling pathway, potentiating the insulin-mediated phosphorylation of the c-Cbl protooncogene. It is only present in mature adipocytes, and its expression is induced upon TZD treatment (287, 288).

These effects of PPARγ in adipose tissue are proposed to be the main mechanism by which TZD improves insulin sensitivity in patients with insulin resistance syndrome. This syndrome is functionally characterized by a poor cellular utilization of glucose, resulting in hyperglycemia, despite an elevated insulin blood level. Obesity and hyperlipidemia are always associated with this syndrome. In such patients, TZDs were shown to be efficient hypolipidemic as well as hypoglycemic agents. A likely mechanism is that increased fatty acid uptake and triglyceride clearance by the adipose tissue redirect fatty acids from the muscle to adipose tissue and thus relieves the fatty acid-mediated inhibition of glucose utilization by muscle cells (reviewed in Ref. 289). PPARγ may also directly affect the insulin signaling pathway in adipose tissue, as suggested by the TZD-mediated induction of CAP. It is also possible that PPARγ in the adipose tissue stimulates the production and secretion of molecules that are regulators of the insulin-signaling pathway in muscle and liver. However, as we will discuss below, the insulin resistance syndrome is also improved by TZD in animals lacking adipose tissue, suggesting that an additional mechanism(s) must be involved.

c. Brown fat vs. white fat: Brown fat, a remarkable heat producer, is best known for affecting the basal metabolic rate, giving protection against cold and regulating energy balance. In agreement with this role, a transgenic mouse in which brown adipose tissue is functionally deleted by a targeted expression of diphtheria toxin A (DTA) has a lower metabolic rate and becomes obese and insulin resistant (290). One characteristic of differentiated brown fat cells is the specific expression of mitochondrial UCP1, which uncouples fuel combustion and ATP synthesis by dissipating the mitochondrial proton (H+) gradient generated by the respiratory chain, producing heat instead of ATP (291). In humans, brown adipose tissue diminishes and/or is dispersed shortly after birth and the role of the human UCP1 gene remains to be defined. However, two other genes related to UCP1 have been described in rodents and humans: UCP2 which is widely expressed (292) and UCP3 which is mainly present in muscle cells (293, 294). Several lines of evidence support the hypothesis that they also play an important role in BMR: 1) identity with UCP1 of 59% and 57%, respectively; 2) down-regulation when access to food is restricted; 3) a correlation between the levels of induced expression and ability of different strains of mice to cope with high-fat diet while remaining lean; and 4) an uncoupling activity demonstrated in yeast or in transfected cells (291). However, up-regulation of UCP2 and UCP3 by total food deprivation may indicate a more complex role, yet to be clarified (295). UCP1-deficient mice obtained by targeted inactivation of the gene are sensitive to cold due to a loss of thermoregulation but, surprisingly, are neither hyperphagic nor obese. This later phenotype may be due to compensation by UCP2, which is ubiquitously expressed and induced in the brown fat of UCP1-deficient mice (296). In view of UCP functions in energy homeostasis, it is legitimate to ask whether PPARs are directly involved in their regulation. A partial answer will be given below.

As for the white adipose tissue, activation of PPARγ is capable of inducing brown adipocyte differentiation from precursor cells (297). Whereas the main regulators of the UCP1 expression are thyroid hormones, β-adrenergic stimulation, and overfeeding, PPARγ-mediated regulation of the UCP1 gene also has been demonstrated using differentiating HIB-1B preadipocytes (298) and in vivo (299). PPARα activation also can induce UCP2 expression in mice liver, but not in BAT, and regulate UCP3 expression in neonatal muscle (300). Interestingly, Puigserver *et al.* (155) showed that the expression of the coactivator PGC1, which can interact with PPARγ but also TR, RAR, and ER, is strongly induced in brown fat during cold exposure. While the data presented in this latter report do not support an important role of the TZD ligand in activating UCP1 through PPARγ/PGC1 interac-

tion, one must not overlook the fact that brown adipose tissue is also characterized by a high level of expression of PPARα, which correlates with high levels of fatty acid oxidation in this tissue. Mice with impaired fatty acid oxidation, through spontaneous and induced mutations in the long-chain and short-chain acyl-CoA dehydrogenase (LCAD and SCAD) genes, are cold sensitive (301). Consistently, PPARα KO mice exhibit a marked decreased body temperature when subjected to fasting (240). Thus, if PPARα-mediated fatty acid oxidation is the pathway preferentially targeted by PGC1, this feature might provide an explanation for the rather poor activity of TZD. Brown adipose tissue is likely to represent a remarkable tool for exploring how PPARα and PPARγ target different genes, and possibly opposite pathways in the same cell population.

d. The adipose tissue as an endocrine tissue linked to the systemic hormonal network: Renewed interest in adipose tissue functions arose with the discovery that adipose tissue actively participates in homeostasis by secreting hormone-like substances such as the TNFα and leptin. Both hormones can be seen as adipostat, in that their synthesis and secretion correlate with the increase of the size of the body fat depot (302, 303).

Production of TNFα by the immune system in response to a tumor or an infection leads to a considerable loss of adipose tissue and a waste of muscle that can result in cachexia. In obesity, the secretion of TNFα by lipid-laden adipocytes also leads to increased lipolysis in adipocytes, generating an increase in circulating levels of FFA, whereas a diminished lipoprotein lipase activity decreases fatty acid uptake and thus decreases lipogenesis. Glycemia is also increased, due to the down-regulated expression of the glucose transporter Glut4 (304). TNFα also counteracts insulin action by altering its signaling cascade (305–307). Direct support for the implication of TNFα in the etiopathogeny of this insulin resistance syndrome comes from TNFα null-mutant mice, which are protected from obesity-induced insulin resistance (308). Paradoxically, however, neutralization of the TNFα receptors in mice results in hyperinsulinemia and decreased insulin sensitivity (309). A study in aging rats also suggests that the parallel increases of the adipose tissue-derived TNF activity and insulin resistance with age are not functionally linked (310), indicating that the pathway between TNFα and insulin resistance is far from being understood.

The antagonism between TNFα and PPARγ appears at three levels. First, as previously mentioned, TNFα is an inhibitor of adipocyte differentiation. These antiadipogenic effects of TNFα most likely result from the down-regulation of PPARγ1 and PPARγ2 expression. This reduction precedes that of other adipocyte marker genes such as aP2 and C/EBPα (311). Reciprocally, the insulin sensitizer TZD effectively opposes TNFα-mediated repression of adipocyte genes (312). Second, PPARγ activators partially reduce TNFα-mediated lipolysis, but not that induced by catecholamines (313). Third, several mechanisms have been proposed that link the role of TNFα in the insulin resistance syndrome to the relief of this pathology through TZD-mediated PPARγ activation. They involve the normalization of TNFα expression in white adipose tissue and in muscle (314), as well as a PPARγ-mediated inhibition of the TNFα-induced hypophosphorylation of the insulin receptor and insulin receptor substrate 1 (315).

Leptin, the product of the ob gene (316), is a 16-kDa protein that is secreted by adipocytes as an indicator of the size of energy stores in the adipose tissue (317, 318). Indeed, high leptin levels in blood were shown to reflect body lipid content in humans and mice (319, 320). A recent study demonstrates that leptin is also expressed by muscle cells in response to hyperglycemia or hyperlipidemia (321). One main target of leptin are the cells of the hypothalamic nuclei through which the hormone triggers both a down-regulation of food intake and an increase in energy expenditure (322). An alteration of this feedback mechanism as it occurs in mutations of the leptin receptor results in leptin resistance (318). It now appears that many other cells, including adipocytes, pancreatic cells, and muscle cells, possess the leptin membrane receptor that is encoded by the *db* gene. In adipocytes, leptin stimulates lipolysis and glucose utilization while in pancreatic β-cells, leptin can decrease the expression and secretion of insulin. These effects can be counteracted by the inhibition of leptin production by TZD-activated PPARγ (323–325). The proposed molecular mechanism for this inhibition implies a functional antagonism between C/EBPα and PPARγ on the leptin promoter activity (326) and is thought to participate in the TZD-mediated improvement of the insulin resistance syndrome.

Difficulties in understanding the respective role of PPARγ, TNFα, and leptin in the integrated pattern of responses to overfeeding and obesity come from observations in mice without fat tissue. In aP2/DTA mice, most of brown and white adipose tissue is progressively deleted via fat-specific expression of the DTA chain (327). Such mice have very low plasma levels of leptin, and thus are hyperphagic, but they do not gain weight. Mice A-ZIP/F-1 express a dominant-negative protein that impairs the function of the transcription factors of both the C/EBP and Jun families and are devoid of white fat throughout development. After an initial period of delayed growth, they gain weight but suffer from steatosis and enlarged organs (269). In both models, the inability to adequately store the ingested energy results in metabolic perturbations reminiscent of diabetes with hyperglycemia, hyperlipidemia, and hyperinsulinemia. Here, the insulin resistance syndrome is unlikely to be due to TNFα and/or leptin production and signaling since adipose tissue is missing. However, in aP2/DTA mice, TZDs are still very efficient in normalizing glucose, lipids, and insulin blood values (328). These observations might be paralleled with results obtained in the Zucker diabetic fatty (ZDF) fa/fa rats. In this strain of rats that carry a mutation in the leptin receptor gene, a progressive obesity occurs with consequences resembling the insulin resistance found in non-insulin-dependent diabetes mellitus. In young animals, hyperinsulinemia compensates for insulin resistance, but when the animals become older, triglycerides overload the pancreatic islet β-cells, which results in a decreased insulin production that may participate in a diabetes decompensation (329). *In vitro*, troglitazone treatment lowers fat content of pancreatic islets isolated from such rats and restores β-cell function (330). Based on these observations, it would be of interest to ana-

lyze aP2/DTA mice at the late stage of the fat deletion process. These animals might also suffer from fat deposits in pancreatic β-cells, whose function, as in fa/fa rats, could possibly be improved by TZD treatment. More interestingly, if troglitazone treatment indeed lowers the fat content of pancreatic islet cells, it might have the same effect in other cells. Overload of skeletal muscle cells with triglycerides and its metabolic consequences, such as reduced glucose utilization, could be corrected by the treatment. Such an adipose tissue-independent mechanism would explain the TZD-mediated improvement of insulin sensitivity in aP2/DTA mice. In support of this line of thought, relatively high PPARγ expression in muscle cells has been observed in obese patients together with a TZD-mediated improvement of the insulin-dependent utilization of glucose by these cells (196). It has also been shown that treatment by TZD of human muscle cells in culture results in an increased expression of the PPARγ protein (331), while an *in vivo* TZD treatment of mice and rats improves the insulin-stimulated glucose uptake in skeletal muscle (332). Alternatively, it is valid to question whether all TZD effects are mediated through PPARγ only, or if other mechanisms are involved. It would not be unreasonable to envisage an action of TZD via a membrane receptor, since some PPAR ligands have a dual mode of action, through membrane and nuclear receptors. Furthermore, a direct or indirect role of the isotype PPARα in the ethiopathogeny of type 2 diabetes mellitus must also be considered. Unger's group observations suggest that activation of PPARα in ZDF fa/fa rats is important for leptin signaling and maintenance of intracellular fatty acid homeostasis in pancreatic islets (179).

In summary, the connections that are appearing between PPAR, TNFα, and leptin signaling might be only the tip of the iceberg of the hormonal control interregulating lipid and glucose homeostasis, from feeding behavior to basal metabolic activity. Progress in the understanding of these regulations will permit innovative and improved therapeutics for type 2 diabetes mellitus which affects massive proportions of the population in industrialized countries.

C. PPARs and control of inflammatory responses

Lipid mediators, particularly eicosanoids such as prostaglandins, leukotrienes, thromboxanes, and lipoxins, are involved in a variety of physiological processes including stimulation or inhibition of inflammation. Therapeutic control of an inflammatory response can be achieved either by blocking the membrane receptors mediating the action of inflammatory molecules or by modulating their metabolic fate through inhibition of their synthesis or stimulation of their breakdown. The first indication of a role of PPAR in controlling inflammation was the demonstration that LTB4, a potent chemotactic inflammatory eicosanoid whose activity is mediated by a membrane receptor (333), also binds to PPARα and induces transcription of genes of the ω- and β-oxidation pathways that can neutralize and degrade LTB4 itself (65). In agreement with the above, dietary n-3 fatty acids and clofibrate, which also bind PPARα, have been reported to accelerate catabolism of LTB4 in granulocytes and macrophages (334, 335). Conversely, PPARα-deficient mice show a prolonged inflammatory response when challenged with LTB4 or its precursor arachidonic acid, possibly due to the absence of stimulation of the catabolic pathways, hence, the increased duration of the inflammation (65). Inhibition of the synthesis of proinflammatory molecules such as interleukin 6 (IL-6) and prostaglandins by activated smooth muscle cells also appears to participate in PPARα-mediated control of inflammation (336), via a decreased activity of NF-κB, a transcription factor regulating cytokine production. Another model is that of mouse aging where the levels of constitutively active NF-κB increase in many tissues and are responsible for an elevated secretion of IL-6 and IL-12. With respect to these parameters, PPARα KO mice age prematurely, have increased NF-κB expression in splenocytes, and present prematurely increased blood levels of constitutive and induced interleukins (337).

Recent studies demonstrate that PPARγ too may have an important impact on inflammation, as treatment of activated macrophages with high doses of the PPARγ ligand 15-deoxy-Δ12,14-PGJ2 provokes a resting phenotype and inhibits the production of the inducible form of nitric oxide synthase and therefore nitric oxide, as well as that of gelatinase B and scavenger receptor A (338). This inhibition is due to an antagonizing activity of PPARγ directed toward the activity of the transcription factors AP-1, STAT, and NF-κB, which are known to control cytokine gene expression. Furthermore, treatment with PPARγ ligands of human monocytes that have been exposed to phorbol ester inhibits the induced expression of TNFα, IL-6, and IL-1β (339). Thus, the antagonism between PPARγ and TNFα, discussed previously for the control of adipocyte differentiation, appears to occur also in inflammatory events. However, the involvement of additional, possibly PPAR-independent mechanisms, cannot be excluded (340, 341).

The inhibition of cytokine production through PPARγ activation might also contribute to the mechanism of action of the nonsteroidal antiinflammatory drugs (NSAIDs). These drugs are known to act by inhibiting cyclooxygenase activity (COX1 and COX2), thus blocking the production of proinflammatory prostaglandins. Indomethacin, a NSAID, also exhibits adipogenic activity at concentrations 100- to 1000-fold higher than that required for inhibition of COX activity. At these concentrations, often required in antiinflammatory treatments, NSAIDs are efficacious activators of PPARγ and PPARα, consistent with their adipogenic and peroxisome proliferator activities (342). Therefore, a possible inhibition of cytokine production by PPAR might explain the incremental therapeutic benefit observed at high doses of these compounds. It is also possible that activation of PPARα at these relatively high drug concentrations contributes, in addition to COX inhibition, to the antiinflammatory, antipyretic, and analgesic properties of NSAIDs through stimulation of oxidative pathways neutralizing eicosanoids, similarly to the mechanism proposed for LTB4. In spite of these links between PPARs and some NSAIDs, it is noteworthy that other drugs of this type do not interact with PPARs, indicating that additional pathways operate for exerting their antiinflammatory properties.

As an interesting complement to these observations, fenofibrate treatment administered to hyperlipidemic patients

not only lowers blood lipid values as previously discussed, but leads to a decrease in the blood of acute-phase proteins, whose levels of expression reflect systemic inflammation (336). This observation suggests that diets that modify PPAR activity and circulating lipid levels might also have a regulatory effect on inflammatory processes.

D. PPARs and atherosclerosis

Atherosclerosis is a pathological process that ultimately leads to the localized obstruction of an artery due to the progressive build-up in the arterial wall of an atheromatous plaque. At least three pathological processes participate in plaque formation: foam cell differentiation, inflammatory reaction, and cell proliferation (343). The passage of monocytes from the luminal endothelial surface to the subendothelial space where they differentiate into macrophages is the initial step. The presence of these resident macrophages in the intima of the vascular wall and high levels of LDL in the blood favor a modification of the LDL particles through oxidation or other poorly defined processes. Endocytosis of these particles by macrophages is then mediated by scavenger receptors. In contrast to the LDL receptor, these receptors are not down-regulated by the intracellular cholesterol content and thus allow an excessive accumulation of intracellular lipids resulting in the formation of lipid-laden foam cells. Cytokines produced by these activated macrophage/foam cells include the macrophage-colony stimulating factor, IL-1, and TNFα, which form the basis of the inflammatory component of the atherosclerotic lesion and promote proliferation of smooth muscle cells. Necrosis of macrophages and lipid-loaded foam cells releases their intracellular contents, resulting in an accumulation of extracellular components that form the fibrous cap of the atheromatous lesion. Eventually, the rupture of this plaque leads to the acute arterial obstruction.

Many aspects of these pathological processes might be modulated by PPARs. We previously discussed the role of PPAR in the adipose differentiation program, which may present similarities with the formation of foam cells. We also presented PPAR-mediated regulation of circulating lipoprotein levels and cholesterol metabolism. In addition, attention has recently been given, using THP1 cells, to the activation of the monocyte-macrophage transition and the concomitant up-regulation of the CD36 scavenger receptor, whose gene is a direct PPAR target. Both phenomenons are under the positive control of PPARγ which is itself up-regulated by oxidized LDL (344, 345). Furthermore, expression of PPARγ has indeed been demonstrated in mouse and human atherosclerotic lesions (345, 346). In contrast to this apparently proatherosclerotic action of PPARγ, inhibition of inflammatory cytokine production by the activated receptor might explain the beneficial effect of TZD in preventing atherosclerotic plaque progression. Similarly, inhibition of the macrophage activities by oxidized LDL (347), whose 9-HODE (9-hydroyxyoctadecadienoic acid) and 13-HODE components are PPARγ ligands, has been observed (348). Obviously, further studies are needed to determine the exact role of PPARγ in the development of atherosclerosis. Proliferation of aortic smooth muscle cells, which express both PPARα and PPARγ, also likely contributes to both atherogenesis and restenosis processes. Activation of PPARγ in these cells leads to a beneficial decrease of the phorbol 12-myristate 13-acetate-induced matrix metalloproteinase gene expression (349). In another study, activation of PPARα by its ligand inhibits COX2 expression and cytokine secretion through repression of AP-1, STAT, and NF-κB signaling (336). It would now be of interest to clarify the respective role *in vivo* of the PPAR isotypes in such cells as well as evaluating possible regulatory roles of PPARs in vascular endothelial cells, where PPARs are also expressed (350–353).

In addition to cell necrosis, programmed cell death by apoptosis occurs in atherosclerotic lesions. Interestingly, treatment of differentiated macrophages with PPAR activators induces an apoptosis that is augmented when the cells are activated with interferon-γ and TNFα (354). PPARγ inhibits the transcriptional activity of the NFκB p65/RelA subunit, suggesting that PPAR activators induce macrophage apoptosis by negatively interfering with the antiapoptotic NFκB signaling pathway. However, it remains to be determined whether PPAR activator-induced apoptosis also occurs *in vivo* in the atherosclerotic lesion and subsequently what the consequences are for plaque formation.

E. PPARs and the development of the fetal epidermal permeability barrier

A particular aspect of lipid physiology, which is of interest with respect to PPAR biology, is found in the skin. The outermost layer of the epidermis, the stratum corneum, contains extracellular lipids delivered by exocytosis of lamellar bodies from epidermal granular cells. After subsequent processing into a matrix of lamellar unit structures, these extracellular lipids provide an efficient hydrophobic barrier to transepidermal water loss. Analysis by *in situ* hybridization of the mouse epidermis during development reveals distinct expression patterns of PPARα, -β, and -γ as follows: in the mouse, PPARβ is already expressed at E11.5, whereas no expression of PPARα or -γ is detected. Once the epidermis is multilayered, PPARα and -γ are expressed in all layers, whereas PPARβ is present mainly in the basal layers. None of the three PPARs are detected in the adult epidermis by *in situ* hybridization (L. Michalik and W. Wahli, unpublished observations). In an *in vitro* model, differentiation of normal human keratinocytes exposed to calcium is accompanied by increased levels of PPARα and PPARγ, whereas the level of PPARβ remains unchanged (355). However, the differentiating medium is very important since PPARβ strongly increases if a treatment with phorbol 12-myristate 13-acetate is used (356). Functional studies have shown that several hormones, including estrogen, glucocorticoid, thyroid hormone, and retinoids, affect epidermal maturation. Overexpression of a dominant negative RAR mutant in suprabasal cells during development results in a thick and loosely packed stratum corneum, which lacks the lipid multilamellar structure and is therefore an inefficient barrier, whereas overexpression in basal cells results in a thin epidermis and dry, scaly skin (357, 358). It is thought that the overexpression of dominant negative RAR makes transcriptionally inactive heterodimers with RXR and might therefore subvert activities of

other RXR partners such as PPARs, TRs, and VDR. Indeed, PPAR ligands such as oleic acid, linoleic acid and clofibrate accelerate epidermal development of fetal skin explants *in vitro*, resulting in mature lipid lamellar membranes forming a functional permeability barrier and a multilayered stratum corneum (359). PPARα activators also promote inhibition of proliferation and stimulate keratinocyte differentiation (360). At the biochemical level, activities of the enzymes steroid sulfatase and β-glucocerebrosidase, linked to the barrier maturation, are increased after treatment with PPAR ligands. Since PPARγ-selective ligands affect neither the development of barrier function nor epidermal morphology, PPARα or PPARβ are more likely to be the isotypes involved, but a direct role remains to be demonstrated. Interestingly, additive effects on the epidermal development of fetal skin explants have been observed between activators of PPAR and the farnesol X-activated receptor (FXR), another binding partner of RXR (359). Both clofibrate and juvenile hormone III, a FXR activator, markedly accelerate fetal epidermal differentiation, sitmulating the expression of both profilaggrin/filaggrin and loricrin, which are structural proteins essential for stratum corneum formation. However, in explants treated with thyroid hormone, glucocorticoids, or estrogens, expression of these genes is also stimulated (361). Together these studies indicate a combined role of several nuclear receptors in epidermal maturation, which include, in addition to the classic ER, GR and TR, the receptors PPAR, RAR, and FXR and their heterodimerization partner RXR.

F. PPARs, carcinogenesis, and control of the cell cycle

A link between PPAR and cancer was first drawn after it became clear that peroxisome proliferators cause a dramatic increase in the incidence of liver tumors in mice and rats. Two major factors, an enhanced cell proliferation and an increased peroxisomal production of H_2O_2, have been implicated (362, 363). Futhermore, nafenopin, a peroxisome proliferator, was shown to inhibit liver cell apoptosis in rat hepatocyte primary cultures, an effect that could also promote carcinogenesis (364, 365). A comparative study of wild-type and PPARα KO mice fed with Wy-14,643 suggest that increased cyclin-dependent kinase 1, cyclin-dependent kinase-4, cyclin D1, and c-*myc* gene expressions might be directly or indirectly PPARα dependent (366). There are marked species differences in response to peroxisome proliferators, with mouse and rat being very prone to peroxisome proliferation, while other species, especially humans, are unresponsive (367). So far, no link has been found between PPAR activators and human hepatocarcinogenesis (368). These species differences could be due to interspecies variations in the expression of PPARα in liver, with levels of expression in humans being 1–10% of those found in mouse and rat (369). An alteration of the PPRE sequence in the human acyl-CoA oxidase gene might also explain the relative human unresponsiveness to PPARα ligands (370). Furthermore, there is evidence for structural polymorphism in hPPARα, but the biological significance of this observation, if any, is unclear. We also previously mentioned species-specific responses to some synthetic PPARα ligands, as analyzed in *Xenopus*, mouse, and human PPARα (67, 371). However, more work is needed to assess how frequently such species differences occur. These differences underscore the care that must be taken when extrapolating results from standard toxicological testing of drugs in rodents to human physiology. Thus, although the PPARα-deficient mice, in which peroxisome proliferation cannot be induced any more, are invaluable for carcinogen bioassays aimed to assess to what extend PPARα is implicated, complementary approaches are wished for. For example, the generation of a mouse expressing hPPARα, would allow comparison of the role of the human protein itself to that of the mouse in a murine background and at murine expression levels.

A certain number of analyses suggest a role for PPARγ in inducing cell growth arrest. In that respect, the physiological model of adipocyte conversion provides a valuable tool to study cell cycle arrest and terminal differentiation. For example, it has been shown that in addition to the coexpression of PPARγ and C/EBP, withdrawal from the cell cycle is required for 3T3-L1 differentiation into adipocyte and involves the hypophosphorylation of the retinoblastoma susceptibility gene product Rb (372). However, activation of PPARγ in Rb−/− mouse embryo fibroblasts is sufficient to induce adipocyte terminal differention (373) and, thus, the link between PPARγ and Rb phosphorylation remains to be established. Cell cycle arrest of logarithmically growing fibroblasts and of SV40 large T antigen-transformed adipogenic HIB1B cells caused by ligand-activated PPARγ have been associated with a loss of DNA binding and loss of activity of the growth-related transcription factor E2F/DP (374). 15-Deoxy-Δ12,14-PGJ2 can also trigger the apoptosis of endothelial cells via a PPAR-dependent pathway (351). Studies based on malignant cells clearly support the concept of PPARγ being implicated in cell cycle withdrawal. Primary human liposarcoma cells, which express high levels of PPARγ (375), can be stimulated to undergo terminal differentiation by treatment with PPARγ ligands or RXR-specific ligands. Simultaneous application of both treatments results in additive stimulation of differentiation, which is characterized by stimulation of adipocyte-specific genes, intracellular lipid accumulation, and withdrawal from the cell cycle (Ref. 375; see also Ref. 376). Activation of PPARγ also induces reduction in growth rate and clonogenic capacity of human breast cancer cells in culture. In one of the breast cancer cell lines, which expresses high levels of PPARγ, the resistance to TZD was associated with a high MAP kinase activity, which might explain a low PPARγ activity due to phosphorylation as discussed previously (377). A similar analysis (378) has demonstrated that the inhibition of MCF7 clonal growth by troglitazone and by all-*trans*-RA is reversible when the compounds are used alone, but becomes irreversible when used in combination. This inhibition is accompanied by lipid accumulation, which, however, is not paralleled by an adipocyte differentiation gene expression pattern, but has been correlated with a profound decrease in bcl-2 gene expression and a marked increase in apoptosis. Interestingly, breast adenocarcinoma tissues from three human patients have responded similarly to the combined treatment when tested in culture (378). Human prostate cancer cells were found to express high levels of PPARγ too, contrasting with the low expression in normal prostate tissue. In clonogenic assays

with these cells, PPARγ activators are efficient antiproliferators (379). Various human colon cancer cell lines express PPARγ at high levels, and addition of a PPARγ ligand not only reduces their clonogenic growth in culture but also decreases their growth when transplanted in nude mice (380, 381). Interestingly, the antiangiogenic effect of PPARγ ligands may also participate to growth inhibition (382).

In vivo studies, however, contrast with these results obtained from cells in culture or transplanted into nude mice. A protumor effect of PPARγ has been recently described in mice bearing a mutation in the adenomatous polyposis Coli tumor suppressor gene. In such mice, treatment with PPARγ agonists significantly increases the frequency and size of colon tumors (185, 186). The discrepancy with the above mentioned results obtained with colon cancer cell lines does not seem to be attributable to the genetic defect that causes the tumors in mice, since some of these lines also bear this specific mutation (381, 383).

In summary, one prominent feature found in the data so far reported is the high expression of PPARγ in tumor cells. If this expression results from an attempt of the cancer cell to down-regulate its proliferative propensity, making PPARγ activation beneficial for controlling the tumor, or from a dysregulated pathway linked to the tumor process, making PPARγ activation an aggravation of the tumor environment, is still a matter of debate.

IV. Conclusions

The discovery of PPARs and identification of fatty acids and their derivatives as ligands, a few years ago, have uncovered an unexpected and fascinating regulatory mode of action of lipids as direct modulators of gene expression. Since then, the excitement has not weakened while compelling evidence has accumulated that PPARα and PPARγ act at crucial nodes of the regulatory network that achieve energy homeostasis in the organism. More specifically, an emerging picture is that of a dual and complementary role of PPARα and -γ isotypes in the regulation of the catabolic and anabolic aspects of lipid metabolism, respectively. Stimulating findings also include the discovery that lipid mediators, such as some eicosanoids (leukotrienes and prostaglandins), are natural PPAR ligands, opening new perspectives for investigating possible novel determinants of energy balance, as well as novel functions for PPARs, with links to glucose homeostasis, cell cycle control, inflammation, and immune response. As a corollary, PPARs are promising targets for therapeutic intervention, through the development of agonists but also antagonists, in disorders such as obesity and diabetes, atherosclerosis, chronic inflammatory diseases, and tumorigenesis.

However, one characteristic of the PPARs is that their activation can occur through a broad spectrum of ligands with rather low affinity. This implies that particular care must be taken when assessing the PPAR dependence of a given signaling pathway. More interestingly, some signals might be transduced by different ways, as exemplified by the subtle interplay between the membrane and nuclear receptors, introducing new levels of complexity in PPAR biology as determinants of the fine tuning of interconnected metabolic processes.

Acknowledgments

We would like to thank Pascal Escher, Daniel Robyr, and Maria Klimek for their help in preparing the figures, and Christoph Meier, Sander Kersten, Nestor Fournier, Bernard Thorens, Sandrine Gremlich, and Liliane Michalik for reading the manuscript and/or for discussions. Furthermore, we are also grateful to Johan Auwerx, Pierre Chambon, Michael Milburn, Tim Willson, Noa Noy, Richard Rachubinski, and Kazuhiko Umesono for communicating results before publication.

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